The Journal of Parasitology

Volume 23 APRIL, 1937 Number 2

BOTHRIOCEPHALUS RARUS N. SP. A CESTODE FROM THE NEWT, *TRITURUS VIRIDESCENS* RAF.*

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Bothriocephalus rarus n. sp. is a tapeworm found in the small intestine of newts, first taken in these little amphibians from Sedge Pool, Douglas Lake, Michigan, the summer of 1927 and later in newts from Fontinalis Run, Burt Lake, Michigan. This same cestode has since been identified by the writer from specimens sent by Mrs. Ruth Shaw Kelley, Kent College, Ohio, which were collected from newts in Seaton's Lake, Uniontown, Pennsylvania, and from newts from an unknown locality in northern South Carolina.

As far as the writer has been able to find in the literature, this is the first mature pseudophyllidean tapeworm to be reported in *T. viridescens* and the second one reported for any amphibian. The first adult pseudophyllidean cestode reported in amphibia is *Chlamydocephalus namaquensis* Cohn 1906, in a small Ethiopian toad, *Xenopus laevis,* from Angra Pequena, Africa. Ortlepp (1926), apparently unaware of Cohn's work, has described from this African toad a tapeworm under the name *Dibothriocephalus xenopi* which appears to be identical.

B. rarus is the only worm of the genus in which the complete life cycle is known (Thomas, 1937), and as in the primitive pseudophyllidean *Archigetes sieboldi* reported by Lueckart (1878) and *Caryophyllaeus nutabilis* Mrázek (1901), there is a fully formed scolex in the procercoid stage. This condition is found in the life cycle of *Cyanthocehalus truncatus* Wolf (1906), which is also a primitive pesudophyllidean. Rosen (1919) calls attention to the fact that *Archigetes* matures in the first host whereas *Cyathocephalus, Abothrium infundibuliformis* and *Caryophyllaeus* require only one intermediate host. Essex (1928) has suggested this in the life cycle of *Bothriocephalus cuspidatus* and the writer finds that in the case of *B. rarus* only the copepod intermediate host is necessary. Neither *Abothrium infundibuliformis* nor *Bothriocephalus cuspidatus* have formed bothria in the procercoid stage. If the

* Contribution from the University of Michigan Biological Station and the Zoological Laboratory of the University of Illinois No. 501.

scolex has any phylogenetic significance then *B. rarus* may represent a pseudophyllidean which in its development is more nearly akin to the family Caryophyllaeidae but in other respects parallels the life cycle of members of the order Tetraphyllidea as is shown in the development of *Ophiotaenia saphena* (Thomas 1931 and 1934).

The following characterization amending Meggitt's (1924) diagnosis of the Ptychobothriidae Lühe 1902 is proposed.

Diagnosis:—Pseudophyllidea: Scolex armed or unarmed with two more or less shallow bothria. Genital pores surficial, dorsal, on opposite surface and posterior to uterine pore. Ovary median, compact, usually bilobed. Eggs undeveloped when laid. Adults in fish or amphibia.

Mammals are omitted as hosts for reasons outlined in the discussion. The morphological features of the eggs are of little taxonomic value for the family diagnosis. The characters for the subfamily Ptychobothriinae Lühe 1899 are the same as those proposed by him except that amphibia are added as hosts.

Since the character of the scolex is of no primary importance as far as the genus is concerned, it follows that it is the same as that for the family. The other characters for the genus *Bothriocephalus* Rud. 1808 follow Meggitt's (1924) diagnosis except the statement concerning segmentation. The proposed diagnosis is as follows:

Diagonsis:—Ptychobothriinae: External segmentation distinct or indistinct. Uterine pore median and ventral; male and female pores median and dorsal. Testes and vitelline follicles continuous throughout the strobilus; the latter in the cortical parenchyma. Receptaculum seminis absent. Beginning of the uterus a winding canal (uterine duct) which opens into a large nearly spherical cavity (uterus sac or uterus s. str.). Adults in fish and amphibia.

Because of the confusion in the literature relating to hosts and to the characterization of the family Tychobothriidae Lühe 1902, the subfamily Tychobothriinae Lühe 1899, and the genus *Bothriocephalus* Rud. 1808–1809 to which it must be ascribed, a detailed account of the worm is given.

Bothriocephalus rarus n.sp.

Specific diagnosis With characters of family and genus as amended. Small delicate cestodes 67 mm. to 300 mm. long when fixed. Scolex 740–912 μ long; 270–378 μ broad in midregion; no neck; with or without prominent terminal disc depending on fixation; bothria shallow. Strobila immediately behind scolex, complete segmentation frequently lacking; mature middle strobila 0.684–1.78 μ long by 0.395–1.16 μ wide; end strobila 0.439–1.66 μ long by 0.353–1.839 μ wide. Segments 60 to 250 at maturity.

Hermaphroditic pore dorsal, nearly median. Ovary compact, bilobular, ventral median 70–175 μ broad by 175–376 μ long at isthmus. Uterine sac ventral, frequently globoidal with eggs bulging anterior and middle third of proglottids. Uterine duct median, in corkscrew spirals. Cirrus sac dorsal, 10.8 by 184.4 μ. Vas deferens irregularly alternates to right and left of median line. Cirrus vestigial. Testes 34 to 62 in medullary parenchyma. Vitelline follicles 210 to 250 in cortical

parenchyma. Cuticula in two layers. Eggs thick shelled, amber colored, operculate, undeveloped when laid. 41–56 μ by 59–65 μ. Procercoid in Cyclops; plerocercoid in newt intestine.

Type host.—*Triturus viridescens* Raf., the vermilion spotted newt.

Location.—Small intestine.

Type locality.—Sedge Pool, Douglas Lake, Cheboygan County, Michigan.

Type date.—June 27, 1927.

Type specimen.—U.S.N.M. Helm. Coll. No. 39511.

EXTERNAL MORPHOLOGY

Bothriocephalus rarus (Fig. 1) has a small scolex with shallow bothria and a rectangular terminal disc; although highly mobile and assuming various shapes in the active living worm (Figs. 2, 3, and 5) the terminal disc is more evident in some preserved specimens than in others (Figs. 7 and 9). The musculature is not so highly developed as in *B. scorpi* or *B. manubriformis,* which it most nearly resembles. The cuticula of two distinct layers, the outer 1 μ, the inner 2.6 μ (Fig. 25), shows numerous pores and canaliculi in the region of the scolex when stained with methylene blue following Kleinenberg's killing fluid (Fig. 20). Segmentation begins directly behind the scolex (Figs. 3 and 9) and frequently the anterior proglottids show an indistinct segmentation between a pronounced transverse furrow as though this separation is of secondary origin. Slight folds may extend through a series of proglottids. It is not unusual for the reproductive organs (Figs. 8, 16, and 15) to begin to appear in the thirtieth segment with uterine pore ventral, in the anterior third and the genital pore dorsal near the posterior third, both near the median line, opening to the surface, in about the fortieth to fiftieth segment. The elliptical uterine pore, regulated by sphincter muscles, varies in mature proglottids from 61.2 μ to 87.5 μ by 96.2 μ to 158.5 μ (Fig. 16). There are only a few eggs in early mature segments but the uterine sacs may later become so distended with them that occasionally the entire anterior and middle third of the proglottids are filled. As many as 285 eggs were counted in ordinary uterine sacs.

In some early segments uterine pores have been found which developed separately and independently of the genital complex. The genital pore (Fig. 12) is on a slight prominence and may be situated either in the posterior part of the middle third or in the anterior part of the posterior third of the proglottid. Mature tapeworms producing eggs may have from 60 to 250 segments, the average number around 129 proglottids. The end strobila are frequently only partially developed.

INTERNAL MORPHOLOGY

The ovarian genital complex appears as a dark staining mass in the posterior third of the proglottid around the thirtieth to fortieth segment (Fig. 8) and occasionally two developmental centers appear in a single

proglottid; the anterior set is usually suppressed. The uterus, at first a more or less straight tube (Fig. 8) soon coils upon itself in corkscrew fashion as development proceeds (Fig. 16) and eggs appear around the sixtieth segment.

From a dorsal view (Figs. 12 and 14) vitelline tubules in varying number are seen to join a common vitelline duct and a rather pronounced vitelline reservoir 54.2 μ by 86.8 μ which is dorsal to and slightly anterior to the compact bilobed ovary. The vitelline reservoir irregularly alternates to the right and the left throughout a series of segments. At its distal end it unites with a fertilization tube emptying into the oötype which is moderately supplied by cells of the shell gland measuring 44.4 μ by 36.8 μ.

The oöcapt, slightly anterior and median to the ovarian lobes, measures 17.3 μ to 21.7 μ in diameter and is supplied with sphincter muscles (Figs. 12 and 23). This muscular swallowing apparatus makes a "U"-shaped bend as it narrows down to form the oviduct, 4.3 μ in diameter. This tube expands again at its juncture with the vagina to 13 μ and continues on as a fertilization tube leading to the oötype, which is 17.3 μ to 21.7 μ in diameter.

The vagina (Figs. 10, 12, and 14) at its juncture with the oviduct expands to 13 μ in diameter for about 75.9 μ of its length, then narrows down to 6.5 μ for about 173.6 μ of its length. It again enlarges to 13 μ as it proceeds anteriorly for a distance of about 65 μ. As it connects with the posterior part of the genital cloaca it constricts to a diameter of 8.6 μ.

A nonprotrusible cirrus empties, anteriorly to the vagina, into the genital cloaca, which opens to the dorsal surface through a short hermaphroditic duct and pore. The vestigial cirrus (Fig. 29) measures approximately 43 μ long by 13 μ wide and continues into the spiral vas deferens enclosed by a weakly muscular ovoidal cirrus-sac measuring about 10.8 μ by 184.4 μ in sections. Where the vas deferens enters the cirrus sac, somewhat posteriorly, its diameter is about 4.3 μ but soon expands into voluminous coiled tubules about 23.8 μ in diameter extending posteriorly almost to the ovarian lobes. The expanded tubules are generally packed with flagellate sperm cells (Fig. 26). The vas deferens irregularly alternates to the right and left in successive proglottids as follows: RLLRRRLRRRLRLLL.

The ovoidal testes (Figs. 8, 16, 24, 25 and 28), 34 to 62 in number, are scattered throughout the medullary parenchyma below the longitudinal muscle threads and are at different levels. They are more numerous lateral to the longitudinal nerve trunks (Fig. 16 and 28) and are continuous proglottid to proglottid. In older segments they are quite apt to be almost entirely spent.

The vitelline follicles (Figs. 15, 16, 25 and 28) ranging from 210 to

750 in number are scattered throughout the medullary cortex, dorsal to the longitudinal muscle threads, in two fields weakly separated dorsally and ventrally in the median line and are continuous proglottid to proglottid. They measure 44 to 55 μ by 39 to 61 μ.

In young worms flame cells can frequently be made out in living specimens under pressure but there seems to be no regular distribution and branching as is present in flukes. The ducts unite in the posterior part of the end proglottid in young worms and empty to the outside through a common terminal pore.

In the scolex (Fig. 21), the excretory tubules form distally a rete mirabile which soon breaks up into four main longitudinal ducts at different levels. By frequent anastomosing and branching, as many as seven ducts (Figs. 8 and 24) may extend through a series of proglottids laterally to median in position.

Ganglionic masses in the scolex (Fig. 21) send back to the proglottids two main longitudinal trunks (Figs. 16 and 28) approximately 13 μ in diameter which are ventral and more median than lateral in position.

Calcareous bodies (Fig. 13) irregular in shape and measuring 8–10 μ by 12–15 μ are scattered rather sparsely in cortical and medullary parenchyma.

The cuticula (Fig. 25), 3.6 μ thick, is really composed of two layers, the outer 1 μ thick, the inner 2.6 μ. The outer layer quickly sloughs off if the cestode is not killed shortly after removing it from the newt's intestine.

DISCUSSION

All of the species mentioned herein as belonging to the genus *Bothriocephalus* are from fish with the exception of *B. rarus*. As the scolex is considered of great importance by some taxonomists and our cestode classifications were primarily based on the character of this organ, it is the first to be considered here.

The scolex in the live *B. rarus* (Fig. 3) when completely contracted loses all evidence of a terminal disc and appears similar to that of *B. claviceps* (Gze.) as figured by Cooper (1918). Corrosive acetic fixation (Figs. 7 and 9) seems to emphasize the terminal disc so that it has in many respects the general appearance of that of *B. occidentalis* as given by Linton (1898). A lateral view of the scolex, Bouin's fixation (Fig. 6), appears much like that figured for *B. formosus* Mueller and Van Cleave (1932). Many cotype specimens in the collection of the writer have a scolex not unlike that of *B. japonicus* Yamaguti 1934. This same author shows a radical departure from these more generalized scolices in that of *B. opsalichthydis* which is more like that of some of the Caryophyllaeidae, Hunter (1927: 172), with loculus-like depressions

in addition to the two bothria. Further variation within the genus is seen in *B. pycnomerus* described by Woodland (1924) as having a crown of minute spines around the terminal disc. A row of pronounced spines is shown in *B. polypteri* (Leyd.) by Monticelli (1890) like a fringe around the terminal disc. We cannot say therefore that there is a generalized scolex which is typical for the genus. In fact there are scolices among other genera which will grade into these, such as that of *Abothrium crassum* of the subfamily Amphicotylinae Lhe.

Southwell (1930) lists the four genera of Lühe (1899) for the family Ptychobothriidae, distinguished as follows:

Bothriocephalus Rud. 1808—uterus sack-like, bothria feeble, receptaculus seminis absent;

Clestobothrium Lühe, 1899—scolex spherical with sunken bothria, receptaculum small;

Phychobothrium Lönn. 1889—uterus a coiled canal, bothria well developed, vitellaria in cortical parenchyma;

Taphrobothrium Lühe 1899—uterus a coiled canal, bothria feeble, vitellaria in medullary parenchyma.

In all the above cases, however, the scolex is not so much the distinguishing feature of the genera as the internal organization of the proglottids. The writer is of the same opinion regarding the scolex as Woodland (1927: 246) and believes "a constant form of ovary or uterus, the presence or absence and the position of the uterine aperture, the disposition of the vitellaria, and the dorsal or ventral position relative to the ovary of the posterior genital ducts" are the fundamental features upon which a classification should be based. The type of scolex may have some weight in distinguishing families as in the case of the Diphyllobothriidae and the Ptychobothriidae.

As one examines the original descriptions of the genus *Bothriocephalus* reported from mammals, as arranged by Meggitt (1924: 122), he is given to doubt the validity of any tapeworms of this genus as parasites of mammals. Meggitt's first case, though questioned by him, *B. andresi* Porta 1911, is quite clearly from Porta's original description and figures a fish tapeworm from the fish *Eucitharus linguatula*. *B. ursi* Landois 1877 was described from a chain of proglottids voided by a bear in a zoological garden. The scolex was not recovered but from the brief account the worm was almost identical with *D. latum*. *B. similis* Krabbe 1865, a cestode of the fox, *Vulpes lagopus,* appears from the author's description to belong to the genus *Diphyllobothrium*. Krabbe's determination was decided by Diesing's (1864) use of *Bothriocephalus* instead of *Dibothrium* Rudolphi. Later the genus *Diphyllobothrium,* with which this last term is by some considered synonymous, came to include only

the features as set forth by Cobbold (1858). Lühe (1899) established the genus *Dibothriocephalus* but there still seems to be doubt as to its priority over *Diphyllobothrium* as Southwell (1930) has indicated. However, there is at present no need for confusing them with the genus *Bothriocephalus*. Other examples from mammalian hosts listed by Meggitt, *B. folius* (*Dibothrium folium* Diesing 1850), in the mongoose, *Herpestes leucurus* and *H. albicandus*; *B. didelphydis* Ariola 1900 in the intestine of the opossum, *Didelphydis marsupialis*; *B. polycalceolus* Ariola 1896 from the seal, *Phoca vitalina,* and *B. sulcatus* in the leopard, *Felis pardus,* all appear to be from the literature in the genus *Diphyllobothrium*. Böhm (1921) likewise has pointed out that Ariola (1900) in his monograph erroneously uses the name *Bothriocephalus* for *Diphyllobothrium*. This same error in many cases is made by Stiles and Hassall (1912). *B. longicollis* Parodi and Widakowich 1917 from the intestine of *Felis yaguarandi* as indicated by these authors "Por lo demas, sus multiples caracteres distinctivos coinciden con la especies *Bothriocephalus latus,*" both from their figures and description (1917: 223–224; 226, Figs. 2, 3, and 4) clearly set forth the fact that they were dealing with a worm of the genus *Diphyllobothrium* closely related to *D. latum*.

Furthermore evidence that the tapeworms of the genus *Bothriocephalus* are parasites of cold-blooded animals lies in the fact that their procercoids, Essex (1928) and Thomas (1936), contain no glands or cuticular spines for tissue penetration such as exist in those of *D. latum, D. erinaces, D. decipiens* Li (1929) and *Ligula intestinalis*. Embryonic stages, such as coracidia, in these two families present characteristic differences. Those of the Ptychobothriidae have more delicate embryophores than those of the Diphyllobothriidae and progress with a jiggling movement whereas the latter after the first few darting movements following hatching settle down to a slow rolling motion like *Volvox*. In the family Haplobothriidae (Thomas, 1930) the cilia are shorter and arranged in rows as in *Paramecium,* the coracidia develop in the eggs while in the uterus so that hatching takes place almost as soon as they are laid. Their movements are swift and darting as that of a *Didinium*. It does not seem unlikely that if more comparative developmental studies were made that the separation of all the families of the Pseudophyllidea might be made easier on the basis of physiological as well as morphological differences in larval stages.

Although Lühe (1902) originally described the family Ptycobothriidae as having thin-shelled non-operculate eggs, this is apparently not a family characteristic as out of ten species of the genus *Bothriocephalus* described from fish, four have been noted with thick brownish-shelled operculate eggs; *B. manubriformis* Linton 1889, *B. cuspidatus* Cooper 1917 (Essex 1928), *B. opsalichthydis* Yamaguti 1934, *B. sciaenae* Yama-

guti 1934; to which may be added the amphibian cestode *B. rarus* Thomas. Others in the same genus that might be mentioned as *B. formosus* Mueller and Van Cleave 1932, *B. japonicus* and *B. acheilognathus* Yamaguti 1934, *B. claviceps* Goeze 1782 (Cooper 1918) have thin nonoperculate shells. On the other hand the eggs of *D. latum* have thick brownish operculate shells as do those of *Ligula intestinalis* with a small polar knob opposite the operculum. This last feature was first pointed out by Landois (1877), later considered by Magath (1919) to be of diagnostic importance but which may be found in the eggs of many other cestodes including those of *B. rarus, B. cuspidatus,* and *B. manubriformis.*

If more attention had been paid by earlier workers to the loosely bilobed reticulate ovary of the Diphyllobothriidae this character would have set them apart from the Ptychobothriidae in which it is compact and more or less bilobed. Southwell (1930: 53) has used this feature together with the position of the pores of the uterus, the cirrus and the vagina in separating the two families.

Within the genus *Bothriocephalus* the vitellaria vary in number and distribution; in *B. rarus,* 210 to 250 in number in two broad lateral fields weakly united on each surface; in *B. claviceps,* 450 to 720 which are not separated into two fields on each surface; in *B. cuspidatus* 800 to 1000 in the cortex and evenly distributed over the dorsal and ventral surfaces. The vitelline reservoir is large in *B. manubriformis* and *B. rarus* but small in *B. scorpi.*

The cirrus sac in *B. manubriformis* is long, cylindrical, at right angles to the surface and with thick heavy walls. In *B. scorpi* it is more spherical, at right angles to the surface and extending only a short distance into the medulla. *B. cuspidatus* has a large sac with thin walls. The medium-sized ovoidal sac of *B. rarus* is thin-walled, at a 90° angle to the surface, and extends half way into the medulla.

The cirrus is short and muscular in *B. manubriformis* and usually not protruded as is likewise the case in *B. scorpi.* The cirrus in *B. cuspidatus* is usually protruded but in *B. rarus* is is vestigial and nonprotrusible.

The vagina of *B. manubriformis* opens directly behind the cirrus or slightly to one side by means of a sphincter bulb. This bulb is lacking in *B. scorpi, B. cuspidatus* and *B. rarus.*

The uterus in *B. manubriformis* and *B. scorpi* is in voluminous folds on either side of the median line, crowding other organs. It irregularly alternates to one side of the median line in *B. cuspidatus* and in *B. rarus* it forms a corkscrew spiral in the median line between the ovary and the uterine sac.

The shell gland is large and close behind the cirrus sac in *B. scorpii.*

It is long and narrow in *B. scorpi* but short in *B. rarus,* in which it lies dorsally behind the cirrus sac and opposite to the coils of the vas deferens.

The spermatozoa (Fig. 26) are somewhat like those reported by Retzius (1909) for *Tetrarhynchus erinaceus.*

The genital pore opens out from a prominent papilla in *B. scorpi* but from only a slight prominence in *B. rarus* whereas there is none in *B. claviceps.* The cloaca and hermaphroditic duct are distinct in *B. manubriformis* but not sharply separated in *B. cuspidatus* and in *B. rarus.*

The number of testes has received more attention by most authors, although this does not appear to be nearly so important as their distribution. *B. formosus* has 30 to 45 testes; *B. rarus,* 34 to 62; *B. scorpii* 30 to 60; *B. claviceps,* 50 to 60; *B. cuspidatus* 50 to 60; *B. manubriformis,* 60 to 70; *B. japonicus,* 60 to 80, and *B. opsalichthydis,* 60 to 100. Cooper (1918) has emphasized the distribution of the testes in *B. cuspidatus* in relation to the nerve cord. In this worm the nerve cords separate the testes into two fields, the inner being much narrower than the outer. This is approximately the condition in *B. rarus.*

In *B. sciaenae* Yamaguti 1934 the testes are in a single layer in the lateral medulla whereas in *B. rarus* they are in different layers.

The lateral nerve cords vary in the different species in position and size within the medullary parenchyma; in *B. manubriformis* they are 18–30 μ in diameter and half way between the lateral and median position of the proglottid; in *B. claviceps* they are 17 μ in diameter and in the dorsal medulla more nearly lateral in position. The nerve cords of *B. rarus* are approximately 13 μ in diameter, more ventral than dorsal and nearer the median than lateral position of the proglottid.

The musculature presents specific differences as does the cuticula. *B. manubriformis* has well-developed longitudinal muscles in bundles; *B. japonicus* has them poorly developed under the cuticula but the inner subcortical muscles are in bundles forming a conspicuous layer; in *B. opsalichthydis* the subcuticular muscles are well developed; the inner longitudinal muscles of *B. scorpii* are in bundles; in *B. claviceps* the inner muscles are not in bundles but are few and weak; in *B. occidentalis* the inner longitudinal muscles are in bundles, those under the cuticula scarce. The longitudinal muscles of *B. rarus* are scarce under the cuticula but immediately under the cortex form a weak layer of scattered fibres. There are also a few scattered but weak transverse fibres.

The cuticula has had little attention other than measurements of its thickness although Lönnberg (1891: 52) mentions that in *B. punctatus* (=*B. scorpii*) there are two layers to the cuticula as is the case with *B. rarus.* The outer layer has pores and canaliculi (Fig. 20) which are obscured by the sloughing of cuticula in specimens that are not promptly killed and fixed as soon as they are removed from a freshly killed newt.

SUMMARY

1. *Bothriocephalus rarus* n. sp. is the only described pseudophyllidean tapeworm found in the newt *Triturus viridescens* and the second one of this order described from any amphibian.

2. This tapeworm appears to be the only one of the genus infecting as final hosts animals other than fish.

3. Its morphology is described and compared in some detail with other Pseudophyllidea in an attempt to discover significant characters for the separation of groups. The scolex and eggs are not entirely suitable for this purpose.

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Explanation of Plates I and II

All figures are camera lucida drawings unless otherwise stated. The projected scale for figures 9, 12, 15, 16, 18, 19, 21, 22, 24, and 28 = 0.20 mm.; for figures 6, 13, 23, 25, and 29 = 0.05 mm.; for figure 1 = 30 mm.; for figures 7 and 17 = 0.15 mm.; for figure 11 = 0.3 mm.

Fig. 1.—The entire worm.
Figs. 2, 3, and 5.—Free-hand sketches of the scolex in successive movements.
Fig. 4.—Anterior segments, dorsal view, showing position of hermaphroditic pore.
Fig. 6.—Lateral view, toto mount of scolex. Bouin's fixation.
Fig. 7.—Surficial view of scolex and terminal disc, toto mount.
Fig. 8.—Early developing proglottid showing in ventral view testes, excretory ducts, genital complex, uterine tube and pore.
Fig. 9.—Scolex, toto mount, showing end view terminal disc.
Fig. 10.—Wax reconstruction, genital complex seen in anterior ventral oblique view.
Fig. 11.—Segmenting operculate egg some time after laying.
Fig. 12.—Surficial view genital complex, toto mount. Hermaphroditic pore dorsal.
Fig. 13.—Calcareous bodies from cortical layer.
Fig. 14.—Genital complex, wax reconstruction, dorsal view.
Fig. 15.—Mature proglottid, toto mount, ventral view.
Fig. 16.—Proglottid more anterior in segments, ventral view.
Fig. 17.—Cross-section through bothria showing four main excretory ducts.
Figs. 18 and 19.—Cross-section through proglottids in region of uterine pore and sac.
Fig. 20.—Surface view of cuticula from scolex; oil immersion.
Fig. 21.—Sagittal section through scolex, showing distribution of ganglionic masses; portions of excretory ducts and nerve trunks.
Fig. 22.—Longitudinal section, cirrus sac and vagina.
Fig. 23.—Section through oöcapt, oviduct, and ovary.
Fig. 24.—Cross-section of proglottid, showing distribution of muscle fibers, ovary, testes, excretory ducts, vitelline duct, nerve cords, and vitellaria.
Fig. 25.—Longitudinal section near cortical medulla, with a testis, excretory duct, muscles, vitellaria, and two layers of cuticula showing.
Fig. 26.—An ovum (left), spermatozoon (center), and vitelline cells (right).
Fig. 27.—Sagittal section through vitelline reservoir, at juncture with oötype and fertilization tube.
Fig. 28.—Sagittal section through entire proglottid, ventral surface.
Fig. 29.—Section through cloacal region and cirrus sac.

PLATE I

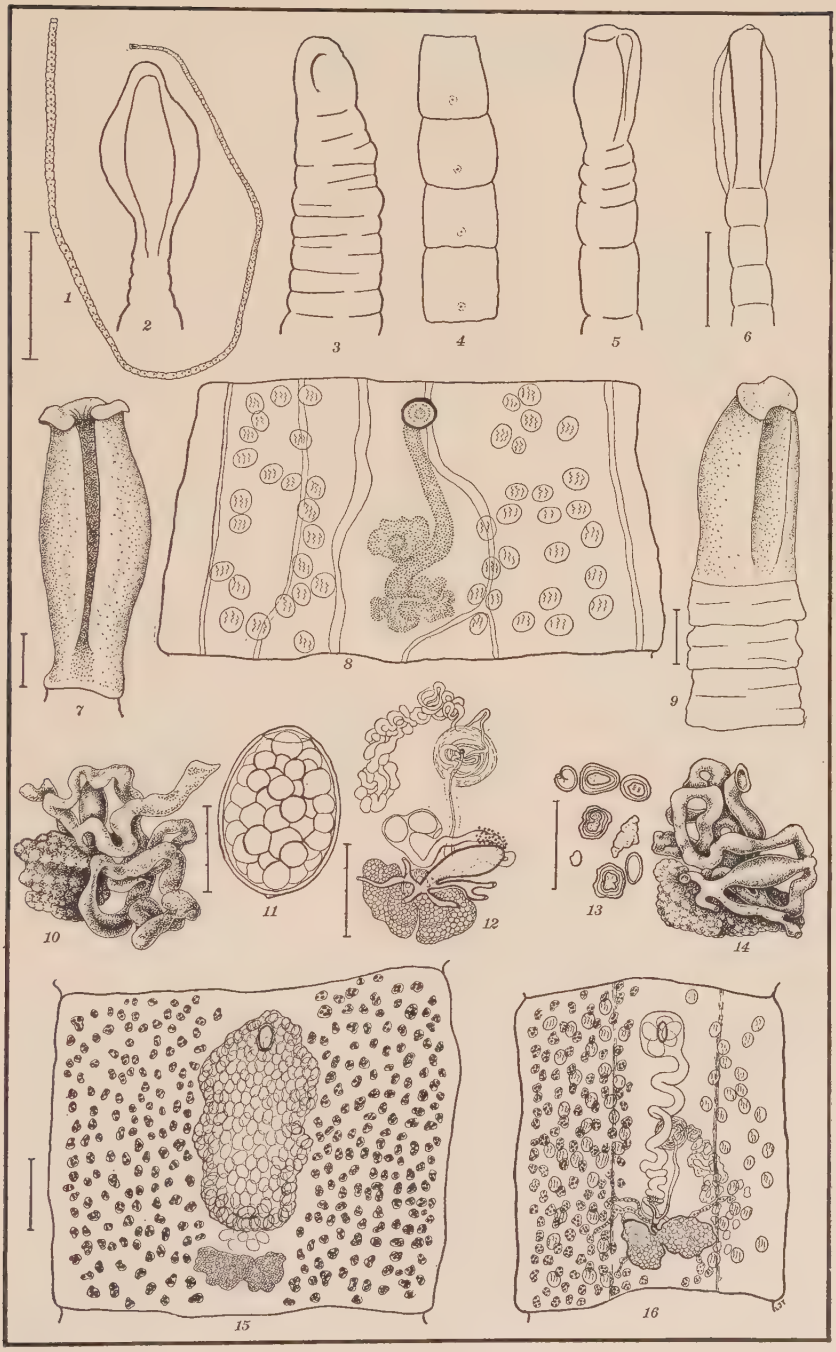
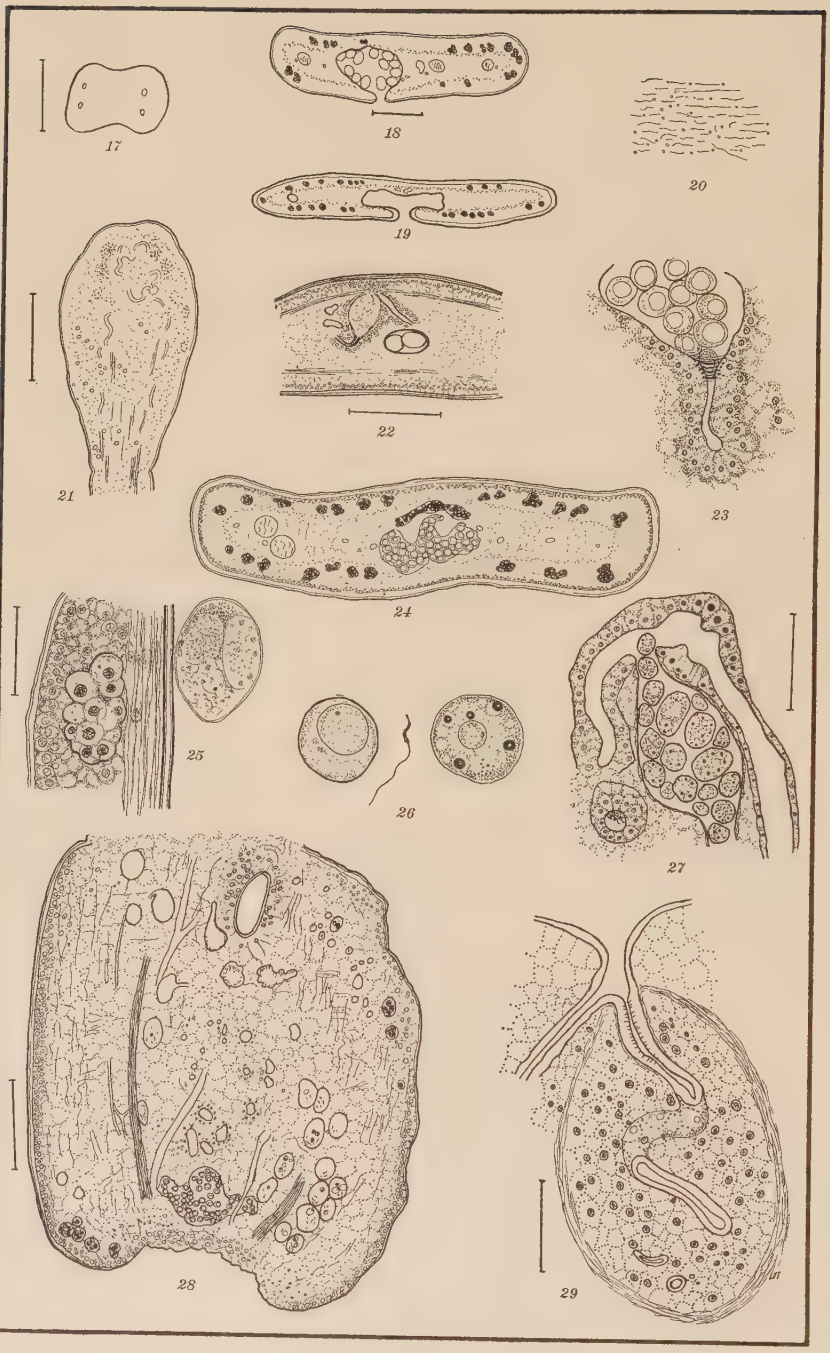


PLATE II



ENVIRONMENTAL RELATIONS AND LIFE HISTORY OF THE TAPEWORM *BOTHRIOCEPHALUS RARUS* THOMAS*

LYELL J. THOMAS

The description and distribution of the tapeworm *Bothriocephalus rarus* has been dealt with in another paper (Thomas, 1937), in which attention is directed to the known range of this parasite which up to the present time has been reported nowhere else but in the small intestine of the vermilion-spotted newts of Sedge Pool, Douglas Lake, Cheboygan County, Michigan; Seaton's Lake, Uniontown, Pennsylvania; and an unknown locality in northern South Carolina.

The tapeworm is uusual in that, to the writer's knowledge, it is the second adult pseudophyllidean tapeworm reported for amphibia and the only one thus far described for the genus *Bothriocephalus* that does not necessarily have a second intermediate host and that has formed bothria in the procercoid stage. These last features are a departure from the classic study by Rosen and Janici (1917–18) on the life history of *Diphyllobothrium latum* and the studies of Li (1929) on *Diphyllobothrium decipiens* and *D. erinacei.* It is also different from the life cycle of *Bothriocephalus cuspidatus* as given by Essex (1928). It, in fact, approaches in some respects the development of *Cyathocephalus truncatus* Pallas as described by Wisniewski (1932*a* and *b*).

The data assembled here are the culmination of experiments made and notes taken during the summers 1927 to 1933 at the University of Michigan Biological Station.

ENVIRONMENTAL RELATIONS BETWEEN THE NEWT AND THE TAPEWORM

In order to carry on life-history experiments successfully, the writer soon found that a careful study of the food and habits of the newt was necessary. Although much has been written about this particular newt, there are numerous contradicting accounts in the literature. Gage (1891), Pope (1924), Noble (1926), Matheson and Hinman (1929), and Morgan and Grierson (1932) gave many helpful suggestions and many of their observations were confirmed by the writer. Grateful acknowledegment is also made to Professor Dougal Brown for information concerning the habitats of *Triturus* in the Douglas Lake region.

It was observed that adult newts had to be taken from Sedge Pool before August first, as after that date most of them had left the pond

* Contributions from the University of Michigan Biological Station and the Zoological Laboratory of the University of Illinois No. 502.

and only occasional individuals might be found. During this migration from the water, there is a remarkable change in the external epithelium, from slimy to dry. Larval newts, 30 mm. in length, were seen leaving the pond from the last week in June up to about August 20, when the Station activities generally ceased. Morgan and Grierson (1932: 61) found *Triturus* larvae in the stomachs of adult newts examined in June, July, and September. Newly hatched newts and eggs were found throughout this entire time; the eggs are yellowish in color with gelatinous capsules, generally attached singly to the vegetation. Attempts were made to hatch and rear parasite-free newts but a series of mishaps prevented the rearing of them in any great numbers. One female in an aquarium laid forty eggs in one day, attached singly to *Elodea,* but during the second night devoured every one. Twenty young newts were hatched from the pond eggs collected in June, 1928, and were used for experimental infections. Twenty-six young were hatched in seventeen days' incubation time at about 75° F. from eggs laid in the laboratory. These were saved for experimental infections. The eggs were laid on vegetation in aquaria June 23, 1929, and hatched July 9, 1929. It was found impracticable to rear young newts with adults in artificial ponds made of wash-tubs as the adults soon ate up the eggs or larvae. During two summers the pool dried up completely by the 20th of August, but young newts were found, with occasional adults, in the moist mud and ooze in muskrat-runs or crowded into small pockets of water teeming with cyclops and other microcrustacea. The food of the larval newts, at least those measuring under 30 mm. in length, consisted principally of microcrustacea, while those 30 mm. or over added insect larvae to their diet.

Adults in the pond lived on aquatic insect larvae, occasional microcrustacea, *Cyclops* and *Cladocera,* water beetles, snails, and small clams, *Sphaerium* and *Piscidium,* molted skins and larval newts. Matheson and Hinman (1929) found that mosquito larvae were preferred by the newts to *Daphnia.* As the pond began to dry up in July, adult newts and larvae with gills atrophied were found in shallow water along the grassy margins, which were also abundantly supplied with microcrustacea. According to Morgan and Grierson (1932) they feed all winter in spring-fed streams with temperatures down to 5° or 10° F.

The "red phase" of the newts is rare in the Douglas Lake region, but four collected by Dr. Charles Creaser and class in herpetology under damp logs near Sedge Pool, July 6, 1931, were given to the author, who placed them in a screened cage with leaf mold and moss until the end of the summer session when they were taken to Urbana, Illinois. They were kept in a screened terrarium at about 72° F. and given an abundant supply of tubificids and *Drosophila* and saved for examination later.

Green vermilion-spotted adult newts found under moist logs had midges, beetles, and earthworms in their intestines.

DEVELOPMENT OF *Bothriocephalus rarus*

The Eggs

Eggs looking very much like those of *Diphyllobothrium latum* pass out of the newt intestine in the feces which are voided in the shape of ovoidal pellets. The eggs are from a light to dark amber color with an operculum about 12 μ in diameter and small thickened knob at the opposite pole (Fig. 15), which is similar to that described for *D. latum* by Magath (1919).

Over one hundred different measurements of living eggs were made between the years 1927 and 1932 which showed an average of 44 μ × 62 μ, with sizes varying between 41 × 59 μ and 56 × 65 μ.

The eggs are laid in an undeveloped condition (Fig. 15) with numerous yolk cells partially obscuring the germ cell which is clear and colorless and appears more or less centrally located; its divisions were followed with difficulty. Eggs were obtained in numbers by placing the tapeworms as soon as they were removed from the newts' intestine in cold well-water. This caused them to be expelled in quantities. Finger bowls and covered two-inch stender dishes with sufficient water in them to cover the eggs were found to be very satisfactory for incubation purposes. In some cases where bacteria coated the eggs, their development was slowed up, but paramecia added to such cultures destroyed the bacteria and cleared the cultures within a few days.

Observations made July 14, 1930, 10:00 A.M., on the normal hatching process showed the onchosphere to be actively moving within the embryophore. Waste granules were most abundant at the pole opposite the operculum. The hooklets were pointed away from the operculum and nearest the knobbed end of the egg. Within the space of ten seconds the operculum was raised, the coracidium oozed out, hooks last; waste granules within the egg were violently agitated. The coracdium seemed to pause the moment it was extricated from the egg shell. The embryophore rounded out, then was extended with the action of the contained onchosphere, rod-like to oval, as the vibrating cilia rapidly carried it away with a few waste granules momentarily adhering. It soon settled down to a vigorous jiggling movement with little progress in any definite direction but eventually approached the surface film. In this same culture dish on July 17, 1930, most of the coracidia were at the bottom of the dish, their embryophores swollen, all ciliary action stopped, yet in a few the onchospheres moved about.

Measurements of one hundred living coracidia, excluding the cilia, taken at random from incubation dishes July 15 to August 1, 1930, varied

between 31 μ × 35 μ and 31 μ × 41 μ. The average measurement was 32 μ × 37 μ. By comparing these coracidia with a similar lot examined two and three days after hatching, variations between 67 μ in diameter and 75 μ in diameter were found, the average for the lot being 70 μ in diameter. This variation in the size of the coracidium was due to the absorption of water by the cells of the embryophore as the onchosphere remained unchanged in size throughout the three-day interval.

Normal and Delayed Hatching of Eggs

At temperatures around 75° F. the eggs develop in about five to eight days, but at temperatures 80° F. to 85° F. the development is speeded up so that they may hatch in as few as three or four days. Eggs expelled from proglottids in warm, shallow water, temperature 85° F., 9:00 P.M. July 19, 1932 were hatching at 9:00 P.M. on July 22.

On two different occasions, July 10 to August 10, 1930, and July 19 to August 15, 1932, eggs were observed that developed very slowly over a period of about a month so that coracidia continued to hatch in the culture dish long after the greater proportion had hatched. This same feature was observed by Thomas (1930) in *D. latum* eggs, but in this mammalian parasite the delay extended over a longer period, November 17, 1929, to January 30, 1930. These were normal coracidia in every respect and the extended developmental period is undoubtedly advantageous in the perpetuation of the species. It was noticed, however, that these delayed coracidia were less opaque than normally hatched ones so that flame cells could readily be seen in the living specimens.

Life Span of the Coracidia

Normally the coracidia are in motion for about three days but during this period their movements become less active as the embryophores begin to swell up with the taking in of water and the ciliary action lags until it ceases altogether. The embryophore may swell to 120 μ in diameter. The onchosphere may continue to move for still another day. Coracidia placed in Ringer's solution modified for crustacea remained active for a week and the onchospheres remained alive for nearly a month. It was discovered that ciliary action could be stopped and started at will by altering the salt concentration of the Ringer's solution.

Morphology of the Coracidia

The newly hatched coracidia (Figs. 13 and 18) average about 50 μ in diameter and their cilia, about 13 μ long, are rather sparsely but uniformly distributed over the spherical embryophore. Nile blue sulphate used as an intravitam stain showed the cilia to be more sparsely distributed than they appeared under ordinary microscopical examination in the living

state. A fine hyalin-like granule appears at the base of each cilium. The outer embryophore is composed of large globular cells of varying size which become highly vacuolated with advancing age. Numerous excretory granules of a yellowish hue are scattered throughout the embryophore. These granules may at times be more or less concentrated in masses around the onchosphere.

The onchosphere is surrounded by a very thin and delicate membrane which appears to be non-cellular. Three pairs of hooks are operated by delicate contractile fibrils and measure as follows: the two outer pairs 10.8 μ long and the inner pair 10.7 μ long. The middle pair measure 3.6 μ from the tip of the hook to the shank. Although muscle cells were noted, they were not worked out in detail as done by Michajlow (1933) for the coracidium of *Triaenophorus nodulosus*. Two flame cells (Fig. 11) below the hooks open by short convoluted ducts through separate excretory pores situated opposite each other. The flame cells continue to beat for some few minutes after they are pressed out of the onchosphere under pressure of the cover slip in Ringer's solution; this same feature was noted by Li (1929) in the onchospheres of *Diphyllobothrium decipiens* and *D. erinacei* also by Thomas (1930) in *Diphyllobothrium latum*. Fine excretory granules are frequently seen in the ducts near the pores and vacuolate cells appear near the flame cells.

INFECTION EXPERIMENTS WITH THE FIRST INTERMEDIATE HOST *Cyclops*

Ingestion of the Coracidia

A number of infection experiments were carried out and repeated each summer to find out in what species of *Cyclops* the procercoids would develop. The *Cyclops* were identified by the late Doctor C. Dwight Marsh and the changes in nomenclature made according to Wilson (1932). Although many species were tried which ate the coracidia, the procercoids failed to undergo development in them and eventually degenerated. *Mesocyclops obsoletus* (= *Cyclops leuckarti*), *Cyclops vulgaris* (= *C. viridis*) and *Macrocyclops annulicornis* (= *Cyclops albidus*) took the coracidia readily and the procercoids developed to maturity in them. Individual traits in these three species were noted, for example, in Experiment (C), Series 1, on July 21, 1927, 8:00 P.M. Two copepods, *C. vulgaris* and *Macrocyclops annulicornis* were placed in a hanging drop with six newly hatched coracidia. When examined the next morning at 8:45 A.M., all six were found to be wandering around in the body cavity of *Cyclops vulgaris*.

This apparent preference of individual *Cyclops* for the coracidia was noted time and again in all three species so that sometimes in concentrated doses, such individuals would get too great an infection and die within a few days. As many as twenty-four procercoids in early stages were

taken from the body cavity of one *Cyclops,* but when the coracidia were diluted, two to six were the most encountered in any one copepod. The copepods ate them as they did protozoa, and the peculiar jiggling movements of the coracidia seemed to attract some individuals more than others. Apparently the embryophore was sloughed off and digested in the intestine shortly after it was ingested because none were seen to be passed in the feces; *Cyclops* dissected shortly after the coracidia were eaten had only a few traces of the old embryophores.

Penetration of the Copepod Intestine

As soon as the onchospheres are free in the digestive tract of the copepods they move into the wall at any point, more especially the intestine, the hooks foremost. When the central pair is extended the lateral pairs are pressed down so that the onchosphere pushes cells aside and thus comes into the body cavity; twenty-five minutes was the shortest time observed in penetrating. Once within the body cavity it extends and contracts intermittently, but the polarity is gradually reversed so that from now on until the maturity of the procercoid the hooks become less active and the opposite end is the progressing part.

Experiment (C).—To Determine the Species of *Cyclops* in which Procercoids Will Develop

Series 1. *CYCLOPS* from Douglas Lake tow.

July 1, 1927.—One hundred *Cyclops* taken at random by spearing with a medicine dropper in very shallow water and examined under the microscope; found negative for tapeworms; placed with coracidia in two two-inch stender dishes.

July 8, 1927.—Six *Cyclops vulgaris* examined had 8, 12, 5, 15, 21 and 7 procercoids respectively with cercomers forming. One *Mesocyclops obsoletus* had three procercoids without cercomers formed; not much development over onchosphere—roll back and forth in body cavity with movements of intestine. *Cyclops vulgaris* with 21 procercoids placed in separate dish.

July 10, 1927.—*Cyclops vulgaris* with 21 procercoids examined but only 8 with fully formed cercomers—all six hooks in cercomers. Three *Mesocyclops obsoletus* with fully formed procercoids. Five *Macrocyclops annulicornis* with fully developed procercoids. Twenty *Cyclops vulgaris* with fully formed procercoids; two with flagellate protozoa in body cavity.

Series 2. *Cyclops* from laboratory culture.

July 21, 1927.—8:00 P.M. Two copepods placed in hanging drop in moist chamber together with six newly hatched coracidia.

July 22, 1927.—8:45 A.M. Coracidia eaten during night. All six found in the body cavity of one copepod identified as *Cyclops vulgaris.* The other copepod was *Macrocyclops annulicornis.*

Series 3. *Cyclops* from Bessy Creek tow.

A

July 1, 1928.—One hundred copepods checked under microscopes for possible tapeworm infection but none found so placed with coracidia in five two-inch stenders.

July 110, 1928.—Forty *Cyclops vulgaris,* two *Macrocyclops annulicornis* and one *Mesocyclops obsoletus* with procercoids. Cercomers beginning to form. Drawings made.

July 13, 1928.—*Mesocyclops obsoletus* found with eight well developed procercoids in the body cavity; they were going through worm-like movements, the end opposite the cercomer would be thrust out, the entire worm lengthen, then contract. Cercomers with all six hooks extend and contract. Drawings made. One onchosphere still in the intestine; food and contractions of the intestine seemed to be forcing it down towards the anus.

July 15, 1928.—*Cyclops vulgaris* examined had ten procercoids in the body cavity. *Mesocyclops obsoletus* three in the body cavity, another *C. vulgaris* had only one in the body cavity.

B

July 17, 1928.—*Mesocyclops obsoletus* placed with 5 coracidia in hanging drop, 9:00 A.M. At 10:00 A.M. all coracidia taken and 5 onchospheres in body cavity.

It is evident from the foregoing series, Experiment (C), that at least three copepods are responsible for the development of procercoids, namely, *Cyclops vulgaris, Mesocyclops obsoletus,* and *Macrocyclops annulicornis.*

Injury to Cyclops

If too many onchospheres penetrate the digestive tract the copepod is apt to die within three or four days. This, however, seemed to be an individual resistance, as some *Cyclops* carried large numbers without difficulty. An occasional *Cyclops* was encountered with flagellate protozoa packing the body cavity and when dissected, three and four procercoids were also found. Whether or not the protozoa were introduced into the body cavity of the copepod at the time of rupture of the intestinal wall during the migration of the onchosphere, is not known.

The Procercoid

Growth of the procercoid.—Within eight to twelve days after infection of the *Cyclops,* the procercoids were mature and ready to infect the next host. In one lot, July 1, 1927, Crustacea were added and a few procercoids were found with cercomers July 8. By July 10 all procercoids had cercomers with the exception of those in heavily infested copepods in which some seemed to lag behind in their development. This was also noted by Essex (1928) in *Bothriocephalus cuspidatus* development, and may be possibly due to the overcrowded condtion or to different intervals at which coracidia were ingested.

On July 18, 1930, a *Mesocyclops obsoletus* was examined from a series infected July 15 and seven procercoids were found which averaged 76 μ × 27 μ when fully extended. Four days later another copepod of this same species was found containing three procercoids averaging 119 μ ×

32 μ. Again on July 27 another *M. obsoletus* examined had three procercoids in the body cavity which averaged 265 μ × 57 μ.

Development in *Cyclops vulgaris* progressed over the same period more slowly; one procercoid in the body cavity measured on July 22, 98 μ × 22 μ; six procercoids in the body cavity of another had the average measurement on July 27, of 108 μ × 32 μ.

Macrocyclops annulicornis had two procercoids which on July 22 measured 130 μ × 22 μ; another on the 27th had three which averaged 108 μ × 32 μ.

Throughout the numerous infection experiments *Mesocyclops obsoletus* seemed best adapted to the maximum development of the procercoids. One of these same copepods dissected in Ringer's solution modified for crustacea had sixteen larval tapeworms five of which averaged 200 μ × 49 μ. The smallest worm measured 163 μ × 43 μ and the largest 217 μ × 65 μ. All of the five specimens had fully formed cercomers but eleven specimens averaging 109 μ × 20.9 μ had no trace of cercomer development. The smallest of these measured 54 μ × 22 μ and the largest 151 μ × 22 μ.

It is interesting to note that in this same series eggs were found in the dish which were still undeveloped on July 29, 1930.

This delayed hatching of some eggs and the ingestion of the coracidia over a period of days could explain variations in the size of procercoids as well as the overcrowding hypothesis.

Bothria—no histolytic glands.—The mature procercoids (Figs. 4, 6, 19, and 20) are without any trace of histolytic glands and the bothria are present as very obvious depressions even while in the body cavity of the copepod. This last feature, at least to the author's knowledge, has not been observed before in developmental studies on Pseudophyllidea belonging to the family Ptychobothriidae.

Excretory canals and flame cells.—The excretory system, consisting of one pair of flame cells and two separate ducts and pores in the hexacanth embryo (Fig. 11), develops in the procercoid (Fig. 20) as two main lateral canals extending the full length and uniting in a common duct as they enter the cercomer. In the scolex the canals anastomose to form a rete mirabile which sends back two branches, one pair ventral, extending a short distance laterally, and the other pair dorsal, extending the full length. No more than two pairs of flame cells have been observed.

The hooks.—The progress of the onchosphere through the intestine of the *Cyclops* is greatly facilitated by means of the six sickle-like hooks which are of two lengths; the middle pair is slightly shorter than the outer pair. They are operated by delicate contractile fibrils which can be demonstrated after the technique of Vogel (1930). Shortly after the

onchosphere has come into the body cavity of the *Cyclops* the hooks lose their active independent movements and are passively brought into the now posterior end by growth of the larvae at the opposite pole which is now destined to become the scolex end. By the eighth day, both in the body cavity and in modified Ringer's solution, there is a tendency for hooks to become separated from the body proper in a spherical constriction known as the cercomer.

The cercomer.—The cercomer is, as noted before, a beginning constriction which includes the hooks at the posterior end of the body; then as a spherical appendage it completely separates the hooks from the body proper by a narrow neck; later it becomes more or less sausage-shaped and quite mobile (Figs. 3, 4, 5, 6, and 20). No procercoids have been observed with hooks outside the cercomer as described by Essex (1927) for *D. latum* and none have been observed with calcareous bodies in this appendage which has great power to elongate but does not constrict off until the procercoid is in the intestine of the second intermediate host (Fig. 22).

Calcareous bodies.—An occasional calcareous granule has been seen even in the onchosphere, but not until the procercoid begins its development do these calcareous bodies become numerous. Some of them, irregular in outline, some bean-shaped or spherical, they frequently present a series of concentric layers of hyalin-like substance which is readily dissolved in weak HCL or acetic acid. They measure in mature proglottids from 11 $\mu \times 9\ \mu$ to 15 $\mu \times 11\ \mu$ and as many as 400 have been counted in one prolottid.

Movements in cyclops.—The procercoids within the body cavity of cyclops are continually extending and contracting themselves and migrate about among the muscles and body organs. In their wanderings they may shift from the abdominal to thoracic region or vice versa. The ordinary movements of the digestive tract of the *Cyclops* may frequently roll them back and forth in the body fluids of the copepod. This is especially true in a *Cyclops* that is not specific for the parasite where degeneration begins within a few days after the entrance of the hexacanth embryo into the body cavity.

INFECTION OF THE FINAL HOST

Natural Infections of Adults Newts

Fifty-three adult newts examined the summer of 1927 had a twenty-five per cent infection. Seventy-four adult newts examined the summer of 1928 showed twenty-six per cent infected with *Bothriocephalus rarus*. Sixty-two adult newts examined between July 7, 1931, and July 11, 1931, had only three tapeworms, or about a five per cent infection. Four "red phase" adult newts taken under logs near Sedge Pool, July 6, 1931, were

examined as follows: No. 1, December 10, 1931, no tapeworms but encysted nematodes, *Spiroxys contortus* and stylet metacercaria, remains of small beetles and Drosophila in the intestine; Nos. 2 and 3, January 4, 1932, encysted nematodes and stylet metacercaria present; No. 4, one *B. rarus* with 165 proglottids, 200 mm. long, reproductive organs beginning to show, uterine pores present, no eggs in uterus as yet. This last newt had molted in the terrarium and was at this time a dark olive color above with characteristic vermilion spots along either side, and belly a golden yellow speckled with black. It was a male with well-developed testes. According to Gage (1891: 1084) the red phase lasts two to three years before the newt returns to the water and becomes viridescent. (Morgan and Grierson, 1932) found molting in some newts all months of the year. Very few newts were taken in the summers of 1929, 1930, and 1932, so as to conserve the local supply.

A shipment of adult newts was received through the courtesy of Professor S. Benton Talbot, Davis and Elkins College, Elkins, West Virginia, April, 1931, who had reported that the newts in a pond near the college were not infested with *B. rarus*. Ten were examined for the parasite but none were found. The others were saved for feeding experiments.

Natural Infections of Larval Newts

Twenty-seven larval newts averaging 30 mm. in length were taken from Sedge Pool July 14, 1928, and examined for tapeworms. Eight of the newts were positive with a total of eleven worms taken. The largest number in any one newt was four. Measurements of the eleven ranged from 223 μ to 4.988 mm. in length. The largest worm had fourteen proglottids. This is typical of subsequent examinations. Only about twenty-eight per cent of the larval newts in nature were infected. This corresponds very closely with the percentage of adult newts infected under natural conditions. The low infection of five per cent recorded for sixty-two newts examined between July 7, 1931, and July 11, 1931, may be explained by the fact that they were all collected about a week before this and placed in a screened crate with partial access to the lake water, and as is noted later, abrupt temperature changes and alterations of the natural environment tend to cause the tapeworms to be shed.

It was noticed that many young newts taken from the pool July 14, 1928, and kept in large uncovered evaporating dishes together with moss and water from the pool, soon after this began to lie partly on the moss with gills exposed to air and then climbed up the side of the dish so that a screen cover was necessary to keep them in. These were a brick red color and corresponded with similar young that were observed leaving the pond at about the same time. It was hoped that they might be carried through the winter to see how their tapeworms fared, but the writer was

doomed to disappointment when early in December of this same year a laboratory assistant unwittingly poured them down the sink.

On July 18, 1930, twelve larval newts were taken from Nichols Bog, near Burt Lake, Michigan. Six measured as follows: 24 mm., 19 mm., 17 mm., 27 mm., 25 mm., and 21 mm., respectively. These were examined for parasites but none were found. Their stomachs were packed with Copepoda and Cladocera. The other six were saved for infection experiments. Two larval newts from Fontinalis Run, Burt Lake, Michigan, July 24, 1933, measuring 32 mm. and 34 mm., respectively, had no tapeworms present. Other examinations of newts from Hook Point on Douglas Lake, Lankester Lake, and Cecil Bay on Lake Michigan were negative for tapeworms.

Experimental Infections of Laboratory-reared Larval Newts

An experiment (LN) was begun on July 30, 1928, to infect laboratory-reared larval newts averaging 22 mm. in length. Twelve newts were selected for this purpose and placed, one each, in two-inch stender dishes to which about twenty infected laboratory-reared copepods in filtered lake water were added. The newts were watched until all the copepods had been eaten and then uninfected *Cyclops* were supplied for food. Six larval newts fed only uninfected Crustacea served as controls.

A newt from this infected lot was examined July 31, 1928, and one larval tapeworm was present in the intestine just behind the pylorus. Apparently the infected *Cyclops* had been ingested recently as the cercomer was just being shed. A camera lucida drawing (Fig. 22) was made of this worm. The following day a newt in dish No. 11 was on its back, very feeble, so was used in experiment (AN). Another found dead in dish No. 2 was examined but no parasites were found. A feeble specimen from dish No. 3 had six plerocercoids in the small intestine which were no larger than procercoids but were without cercomers.

Again on August 2 a very feeble newt in dish No. 7 and two plerocercoids somewhat larger than procercoids were found. Newt in dish No. 4 seemed sluggish and anemic so it was used in experiment (AN). The next day another newt in dish No. 3 seemed very inactive so it was examined and a worm with a very mobile scolex but no sign of proglottid formation was taken from the upper end of the small intestine. The newt in dish No. 9 was dead and one plerocercoid dissected out was used in experiment (G). The larval newt in dish No. 12 was used in experiment (AN), August 5. As the remaining newts were very weak it was decided to close the experiment on August 5. Ten larval tapeworms, all near the pyloric end of the small intestine, were taken from the newt in dish No. 6. These were all active specimens and were used in experiment (G). The newts in dishes Nos. 8 and 10 were used in

experiment (AN). The six controls were also examined at this time and no tapeworms were found.

It was quite evident from this experiment (LN) that the larval newts obtained their infection as in nature from eating infected *Cyclops*. Six out of seven directly examined were positive and the presence of tapeworms seemed to be associated with a weakened anemic condition which was not apparent in the controls.

A second series of twelve larval newts was infected on July 15, 1929, with the idea of keeping the larvae until they were adults. These were taken to the University of Illinois, Urbana, Illinois, at the close of the Biological Station, where only two survived an overdose of chlorine in the university water supply. These two were kept in a terrarium with access to pond water or land until January 30, 1930. At that time they were 60 mm. long, without gills, yellowish red in color. One found dead on this date had one *B. rarus* with 165 proglottids. The other *Triturus* was killed and examined and a tapeworm with 140 segments was removed from the small intestine. Reproductive organs were beginning to appear in the end strobila of both specimens.

In order to check again the pathological effects of the worms on larval newts, six larvae were infected on July 18, 1930. One was found on August 10 on its back with blood barely moving in the gill filaments. The other five were very sluggish compared with the six controls so they were all examined for tapeworms. The six infected newts had the following number of worms, respectively: No. 1, four; No. 2, two; No. 3, twelve (this one found on its back and very feeble); No. 4, fifteen; No. 5, nine, and No. 6, three. The presence of tapeworms was undoubtedly the chief factor in producing the feeble condition of larval newts.

Experimental Infections of Adult Newts

An experiment (AN) was set up to determine if adult newts may become infected by eating larval newts. As no laboratory-raised adult newts were available, specimens from Sedge Pool were isolated for a week in finger bowls and their feces checked for a week to determine if they already harbored egg-producing worms. The newts were fed daily bits of fish or frogs or very young snails. Twelve were selected in this manner and six of them served as controls. Later, when specimens from an uninfected source, Davis and Elkins College, West Virginia, were available, a second series was tried.

In the first series from Sedge Pool a feeble larval newt from an infected lot was placed with an adult August 1, 1928. As it was eaten, the adult was fed another the following day. Its behavior was watched. The adult approached, creeping like a cat, its tail moving from side to side. When it was almost within striking distance it snapped but missed.

Following its prey it suddenly grabbed the young newt by the head and began gulping it down, the tail disappearing last. The adult was examined August 6 and an adult tapeworm about 200 mm. long with all mature proglottids shed (apparently from a previous infection) and five juvenile tapeworms similar to those from the young newts; also eight adult flukes, *Brachycoelium hospitale,* were present in the small intestine.

Again on August 4, 1928, another adult was given a larval newt. Sometime during the night of August 5 it was eaten but when examined the morning of August 6 no parasites were found.

On August 5 four more adult newts were given larval newts from infected stock. One examined on August 6 had one plerocercoid and a tapeworm with five proglottids. Another examined on August 7 had six plerocercoids. The other two examined this same day had no worms present. It was then thought that better results might be obtained by direct feeding of plerocercoids.

Direct feeding by means of a pipette was tried on August 10, 1928. Four plerocercoids were given by mouth and the adult newt was examined the following day. One plerocercoid was found in the small intestine. One was recovered from another adult given six plerocercoids. This direct feeding was not as successful as had been anticipated. Possibly the plerocercoids could not survive the stomach juices as well as when protected by the body tissues of the larval newt.

With parasite-free newts available in 1931, ten adult newts were fed a larval newt each from infected stock, and ten were used as controls, August 4, 1931. The entire lot was examined on August 11 with the following results: Nos. 2, 5, 6, and 10, no tapeworms; Nos. 3, 7, and 9, one tapeworm each; No. 1, three worms; No. 4, five worms, and No. 8, two worms. The controls were negative.

After this last experiment only one of the West Virginia newts from Davis and Elkins College remained. This one was placed in a finger bowl containing numerous infected *Cyclops* to see if adult newts become infected by eating infected *Cyclops* directly. It was observed snapping up the copepods August 4, 1931, and when it was examined on August 11 fourteen plerocercoids were taken from the duodenal end of the small intestine. Just this one case was tried experimentally but it would seem to indicate that the direct method of infection in adult newts is possible.

Conclusions from the Foregoing Experiments LN and AN, and Notes

1. Larval newts become infected with *Bothriocephalus rarus* by eating infected *Cyclops.*

2. Adult newts may become infected with the tapeworm by eating infected larval newts.

3. Adult newts may become infected by eating parasitized copepods directly.

4. "Red phase" newts evidently carry their infection from the pond as larval newts until they return to the pond again after many months. This was true of the red newt taken July 6, 1931, on land and kept in a terrarium until it changed color; it also applies to the larval newts reared in the laboratory and infected July 15, 1929, experiment (LN), Series 2, and found harboring tapeworms in January.

OBSERVATIONS ON SHEDDING OF PROGLOTTIDS AND DEGENERATION OF THE TAPEWORM IN ADULT NEWTS

It is well to call attention to the fact that adult newts readily shed their tapeworms when in captivity, especially with sharp changes in the water temperature in which they are kept. On several occasions the newts were observed to swallow again the live tapeworms that had been passed from their intestines. In each of three adults examined one hour after this performance only partly digested worms were found in the stomach. On June 26, 1927, an adult newt in an aquarium was observed to void three segments of the tapeworm which were rolled in a pellet the same as the feces, and covered with a tough mucus. These pellets, teased apart, were found to contain mature proglottids filled with eggs, which developed normally and hatched normal coracidia within eight days. Such a sealed mucus-covered packet is well-adapted to delay hatching and release a concentrated mass of eggs upon disintegration of the mucus.

Two adult newts taken July 6, 1931, positive for tapeworms as shown by fecal examination, were placed in aquaria without food and when dissected July 20, 1931, had one worm each with scolex and a few undeveloped proglottids. Portions of the worms were noted from time to time in fecal pellets.

Three adult newts positive for tapeworms July 7, 1932, were placed in a covered aquarium without food and at the close of the Station were taken to Urbana, Illinois, where on September 10, 1932, they were examined. Each newt had but a single tapeworm with only a few undeveloped segments. During all this time no food was given to them.

In the normal environment pond, Sedge Pool, the egg-laying season extended, as far as my observations go, from June to August, with larval newts leaving the pond from the latter part of June into August and undoubtedly later. Of the youngest stages found in August, there is the possibility that they did not leave the pond as the colder temperatures of fall came on, and remained in the pond until the following spring or summer after nearly a year in the water. From the observations of Morgan and Grierson (1932) newts feed throughout the winter months

even under the ice. Food is essential for normal tapeworm development as the author has observed in the tapeworm *Haplobothrium globuliforme*, of *Amia calva*. Fresh-water dogfish taken throughout the winter months had very little food in their digestive tracts and accordingly only undeveloped worms were present until the active feeding in the spring and development of the gonads. The author, Thomas (1934), has noticed in frog tadpoles that larval tapeworms taken in by eating infected *Cyclops* do not come to maturity until the tadpole metamorphoses into a frog and likewise changes its food habit. There is no evidence as yet that the tapeworms have normal egg production during the land phase of the newt. It is known that starvation or abrupt temperature changes cause the shedding of mature proglottids. The known food habits of *Triturus* throughout the year leads one to expect to find mature worms at any season of the year in individuals living in the water. The slow development of the tapeworms in experimental larval newts may have been due to deficiencies in their normal diet.

ATTEMPTS TO CULTURE LARVAL TAPEWORMS

Because the juvenile tapeworms of *B. rarus* seemed normal in Ringer's solution, lived for several hours in the intestine of the dead host, and were from cold-blooded animals, the possibility of continuing their growth in culture media was suggested. Summarized, the experiment (G) was as follows: On August 5, 1928, plerocercoids from the intestines of larval newts were placed in test tubes with about 20 cc. of Ringer's solution modified for amphibia. The tubes were kept in cold water to maintain them at as uniformly low temperatures as possible. To some of them glucose was added to make a 0.03 per cent solution. To others a drop of boiled hay infusion was added. The solutions were changed to fresh ones every other day. At the close of the Biological Station they were continued at the University of Illinois. Those supplied with hay infusion remained alive and active until October 2, 1928. Two flukes, *Brachycoelium hospitale*, were likewise kept in this solution for the same period.

From the foregoing experiments, the writer believes that given the right amino-acids, intestinal worms may be cultured just as it is possible at present to culture tissues of the vertebrates. With worms from cold-blooded animals living in such a complex environment as the intestinal lumen, the temperature factor, if low, is plainly not the most important one.

DISCUSSION

The life cycle of *Bothriocephalus rarus* as determined by the foregoing experiments and observations is as follows: The eggs passed in the feces of adult newts hatch within three to eight days in water a ciliated coracidium, depending upon the temperature, development being

more rapid at higher temperatures. The ciliated coracidia active for about three days tend to congregate near the surface film but gradually swell up and sink to the substratum. During the three days they may be eaten whole by a copepod and within thirty minutes may penetrate into the body cavity. Here polarity is reversed so that the end opposite the larval hooks becomes the progressing end and the six hooks within ten days are constricted off into an appendage, the cercomer. The procercoid is now fully formed and ready for the next host, the larval newt. In *Cyclops vulgaris, Macrocyclops annulicornis* and *Mesocyclops obsoletus,* development is completed normally within two weeks. The larval newts concentrated in shallow water around the grassy margins of the pond where adult newts as well as copepods are abundant, find numerous infected *Cyclops* in their normal diet of microcrustacea. The cercomer of the procercoid is shed in the intestine of the newt soon after it is ingested. Development from now on seems to keep pace with the development of the newt. (Thyroxin feeding at this point might be of interest to determine the rate of tapeworm development correlated with the newt development.)

It is not known exactly how long they may be carried in the "red phase" or in the green land phase or whether or not the tapeworm comes to maturity in larval newts that may stay in the water for nearly a year. Gage (1891: 1084) says that the newt may remain in the red phase from two to three years. It is known that larval newts hatched early in July and infected, when kept in aquaria, were not ready to leave the water until December, and two, experiment (LN), Series 2, kept until January, showed worms almost mature. Under natural conditions the newts hatched in June or July when around 30 mm. long were found leaving the pond and would wriggle off into the damp moss. It was also observed that larval newts collected from the pond and about 30 mm. in length were soon ready to leave evaporating dishes in which they had been placed. It is evident, however, that the tapeworm may undergo a period of rather slow development in the second intermediate host so that there is no need of a third host. On the other hand, the adult newt may become a third host by devouring an infected larval newt. There is also the possibility of direct infection of adult newts by eating infected *Cyclops,* although only one case is offered as evidence for this. The appearance of bothria in the procercoid and the absence of hystolytic glands or penetrating spines seems to further substantiate the evidence at hand that only one intermediate host, the copepod, is essential in the life history. This reduction in intermediate hosts is much along the same line of development as Wisniewski (1932 a and b) has described for *Cyathocephalus.* Whether or not this is a return to a primitive condition or a degenerate one is not in the province of the author to settle. Furthermore, the members of the genus *Bothriocephalus* do not appear

from their known life-cycles (Essex 1928) and the present paper, to be parasites of warm-blooded animals.

SUMMARY

1. Eggs of *Bothriocephalus rarus* Thomas develop and hatch in three to eight days depending on temperature.

2. Coracidia ingested whole by *Mesocyclops obsoletus, Cyclops vulgaris,* or *Macrocyclops annulicornis* may penetrate the digestive tract and come into the body cavity within thirty minutes.

3. The procercoid may develop ready for the next host, a newt, within ten to fourteen days.

4. Bothria appear in the procercoid.

5. Either a larval or adult *Triturus viridescens* may serve as the final host by ingesting an infected *Cyclops.*

6. The cercomer is lost in the intestine of the newt shortly after the infected *Cyclops* is eaten.

7. Adult newts may become infected by eating infected larval newts.

8. The "red phase" or green land phase of *Triturus* may carry the infection over from the pond phase or larval stage of the newt back to the pond again.

9. Adult green newts which migrate from the pond may also distribute the tapeworm infection to other ponds or back to the same pond.

10. Larval tapeworms from the intestines of newts were kept alive in culture media without apparent growth for nearly two months.

11. Starvation inhibits proglottid formation and rapid temperature changes may cause the host to void the tapeworm.

12. Heavily infected larval newts become anemic and die.

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Description of Plates I and II

The drawings, with few exceptions, were made with the aid of a camera lucida from live material. The projected scale for figures 1–6, 15–21, and 25 = 0.05 mm.; for figures 7, 14, 19, 22, 23, 24, 26 and 27=0.20 mm.; and for figures 8– 11=0.02 mm.

Figs. 1 and 2.—Procercoids from the body cavity of cyclops, in normal saline solution.

Figs. 3, 4, and 5.—Procercoids removed from the body cavity of cyclops, in Ringer's solution modified for amphibia.

Fig. 6.—Procercoid from the same lot as figs. 3, 4, and 5 showing early construction of cercomer.

Fig. 7.—Development of procercoids after seven days in cyclops.

Fig. 8.—Coracidium in egg about to hatch.

Fig. 9.—Hexacanth embryo showing granules in the excretory system as in *D. latum*.

Fig. 10.—Hooklets beginning to show in onchosphere after two days' development in egg.

Fig. 11.—Onchosphere showing flame cells, ducts, and muscles.

Fig. 12.—Coracidium hatching—free-hand sketch.

Fig. 13—Coracidium.

Fig. 14.—Development of procercoids after five days in cyclops.

Fig. 15.—Egg, shortly after being laid.

Fig. 16.—Development in egg after three days at 75° F.

Fig. 17.—Egg shell, operculum open, serrated margin of opening showing.

Fig. 18.—Coracidium one day after hatching.

Fig. 19.—Six mature procercoids in body cavity of cyclops after ten days' development.

Fig. 20.—Procercoid from body cavity of *Cyclops vulgaris* in Ringer's solution, showing bothria and excretory canals.

Fig. 21.—End view of scolex of procercoid in Fig. 20.

Fig. 22.—Procercoid, shortly after it had been ingested by experimental newt, remnant of cercomer present.

Fig. 23.—Procercoid after six days in Ringer's solution modified for amphibia.

Fig. 24.—Another view of the same specimen as in Fig. 24.

Fig. 25.—Young tapeworm from intestine of experimental newt.

Fig. 26.—Young tapeworm from larval newt (experimental).

Fig. 27.—Young tapeworm from 30 mm. Sedge Pool newt Aug. 10, 1928.

Fig. 28.—Free-hand sketch of young tapeworm attached to intestine of experimental newt.

PLATE I

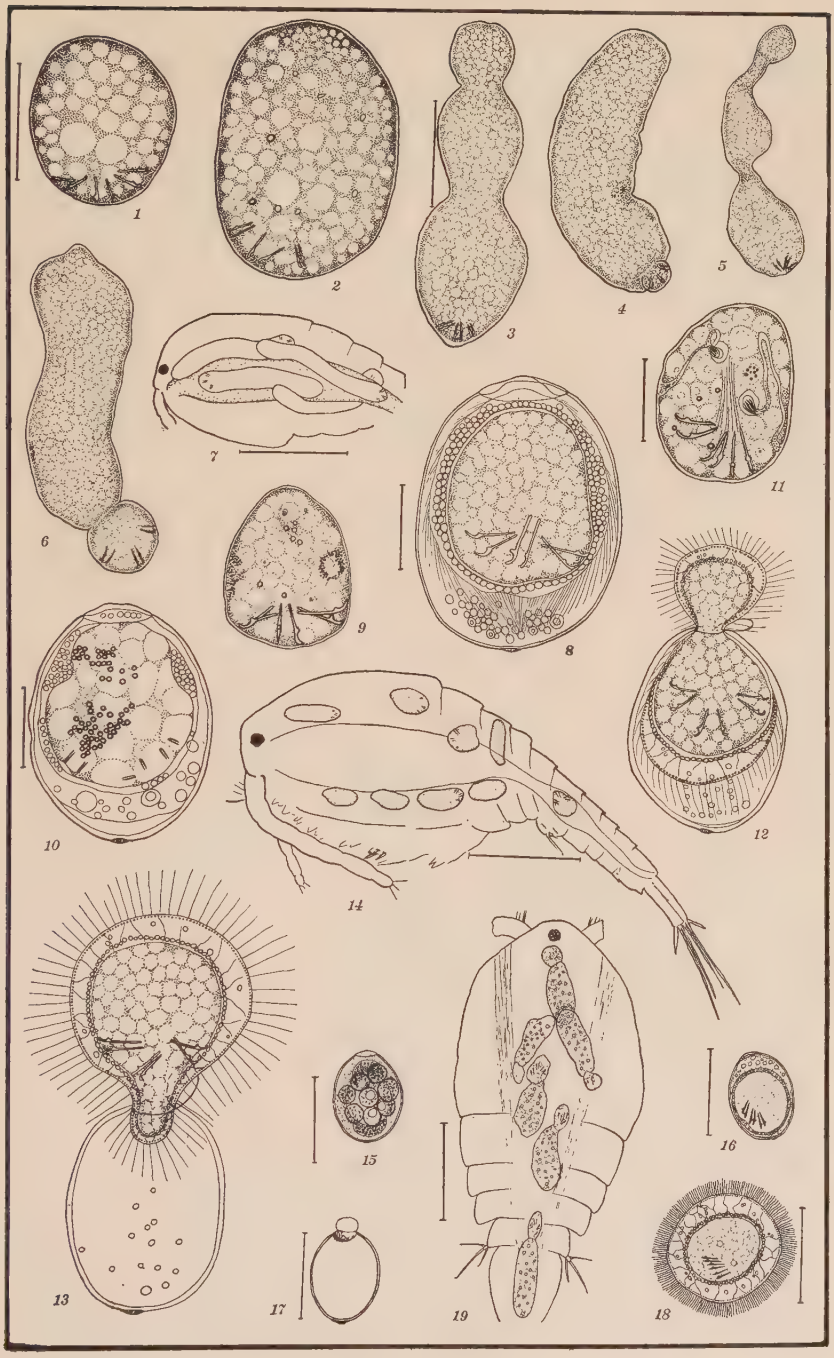

PLATE II

MODIFYING THE QUANTITATIVE CHARACTER OF COCCIDIAN INFECTION THROUGH A DIETARY FACTOR*

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It seems reasonably safe to say that until recently the only clearly demonstrated methods of modifying the quantitative character of coccidian infection were concerned with the size of the infective dose and the immunizing effects of previous infection (1, 2). After investigation (2) had indicated the ineffectiveness of (1) attempts to establish a state of passive immunity in the receptive animal through the transfer of immune sera and tissue extracts, (2) injection of coccidian protein to stimulate the host's resistance to the parasite's development in its body, and (3) splenectomy and intercurrent *Bartonella* infection, attention was directed to possible dietary relationships. These studies (3, 4) brought out that no significant changes in numbers of terminal stages (oöcysts) of coccidia discharged by the host could be produced by such measures as general vitamin and mineral deficiency, vitamin-A deficiency, vitamin-D deficiency, high-casein diet, high-salt diet, low-salt diet, skim-milk supplements, tobacco dust supplements, and iodine or vinegar in the drinking water. In all these experiments the laboratory rat was employed as the host, and *Eimeria miyairii* as the parasite.

Curiously enough, it was discovered (3, 5) that a host which had been on a diet deficient in both vitamins B and G for 9 or 10 days previous to the date of experimental infection would discharge but a fourth or fifth as many oöcysts as one which had received an adequate diet, although infective doses of the microörganism were the same in both cases. Following up this first experimental modification of coccidian infection by means other than manipulating the infective dosage and previous infection, it was demonstrated (6) that at least most of the observed effect could be ascribed to vitamin-G deficiency alone.

It was plain during this preliminary work that the failure of the coccidium to make the expected numerical increase in the intestine of its host was due to the lack of something in the diet. The addition of powdered yeast, either autoclaved or unautoclaved, to the vitamin-G deficient diet to the extent of 10 per cent supplied the missing factor and favored the parasite's normal development. Naturally, the young rat host on the deficient diet does not make normal growth and suffers in other respects. In the hope that there might be located sources of vitamin G that would

* Supported in part by grants from the Industrial Science Research Fund of Iowa State College and from the American Academy of Arts and Sciences.

favor the growth of the host and simultaneously retard that of the coccidium, attention was directed towards pork liver and powdered skim-milk, materials widely used by experimenters as sources of the elements constituting the vitamin-G complex. Briefly, it was learned (7) that when the basal diet was supplemented each day with 1 cc. of an alcoholic extract equivalent to 4 gm. of pork liver, the number of oöcysts discharged was about a fourth of normal and the rat made but little growth. When the supplement consisted of 2.0 per cent powdered liver intended as a source of vitamin G and 2.5 per cent rice polish intended as a source of vitamin B, fair growth gains were made by the host, and yet the number of oöcysts discharged was only about an eighth that from the controls on an adequate diet.

FURTHER EXPERIMENTS WITH LIVER AS A SOURCE OF VITAMIN G

First trial.—The experiment was so designed that a comparison might be made between the numbers of oöcysts passed by rats on an adequate diet with powdered yeast as the source of vitamins B and G, and those passed by animals also on an adequate diet with liver as the source of vitamin G and rice polish as the source of vitamin B. Rice polish is known to contain also some vitamin G, but it is much richer in B (8). The ration for the control or reference series was made up by parts as follows: beet sugar, 60; casein, acid-water and alcohol extracted, 18; cellulose flour, 3; butter, 4; cod-liver oil, 1; salt mixture, 4; Fleischmann's powdered yeast, 10. The ration for the test series was the same as the foregoing except that powdered pork liver was substituted for the yeast, and 4 parts of the sugar were substituted with rice polish. The powdered liver was made by drying the ground-up fresh liver in a warm room under an electric fan, and powdering the dry product in a mill.

The rats were placed on the experimental diets from nine to eleven days before experimental infection was commenced, and kept on them until about 3 or 4 days before the period of discharge of oöcysts was expected to expire. Then they were placed on the growing ration to flush the oöcysts from the intestine. Infection was accomplished in the case of rats by injecting into the stomach through a catheter 1500 sporulated oöcysts of *Eimeria nieschulzi* (= *Eimeria miyairii*) on each of three days within a period of five consecutive days. As soon as the discharge of oöcysts commenced, the rats were placed in cages with hardware cloth bottoms and over pans holding a liquid disinfectant (a 0.5 per cent solution of "Kresol" dip). At intervals the pellets were thoroughly disintegrated with a masher and an electric mixer, the total collection diluted to a known degree, and counts made of the oöcysts in the diluted feces by means of a haemocytometer. Thus it was possible to ascertain ap-

proximately the total number of oöcysts in the collecting pan. Counts were made until elimination of oöcysts had practically ceased.

It should be stated that the rats belonged to the famous Wistar A inbred strain. The animals employed on the experiment were raised in the laboratory, and frequent examination of the pellets showed that they had not had an infection prior to the intentional infection. This precaution assured absence of partial immunity. The animals in test and reference series were from divided litters, and not different litters.

The 9 rats on the liver diet each discharged millions of oöcysts as follows: 23, 40, 44, 100, 12, 30, 33, 39, 35; mean, 39.56. Those on the reference diet: 185, 176, 85, 99, 159, 76, 174, 137, 148; mean, 137.67. The mean for the test series is about 0.29 that of the reference series. The difference of the means is 98.11. When this difference was tested by Fisher's small sample method (9), a *t*-value of 6.1 was obtained, whereas 2.921 is the value that appears in the table for 16 degrees of freedom and a probability of 0.01; the difference between the means is, then, highly significant.

It is of interest that both series of rats were immune to reinfection. It is of further interest that both series made excellent growth during the period on the diets. The test series increased in mean weight from 75.8 gm. to 147.8 gm. during the 22 days liver was fed as the principal source of vitamin G; net gain, 72.0 gm. The reference series increased in weight from 78.4 gm. to 150.3 gm. during the 22 days yeast was fed as the sole source of vitamin G; net gain, 72.0 gm. By a mere coincidence, the net gains made by the two series were almost exactly the same. It might have been desirable to have carried the animals longer on their respective diets, but the immediate purposes of the experiment were served by the shorter period. Likewise, it will require further investigation to ascertain whether it is necessary to leave the animals on the liver diet as long as 9 to 11 days before the first experimental infection, and whether a longer preliminary period on the diets might create a still greater spread between the mean oöcyst counts of the two series.

Second trial.—Five rats that had been on the reference or yeast-containing diet eliminated oöcysts, in millions, as follows: 362, 208, 171, 115, 200; mean, 211.2. The test series received the same diet as in the first trial, except that instead of 10 gm. powdered liver, liver extract (7) equivalent to 10 gm. fresh pork liver was dried onto the sugar. The 5 animals receiving liver extract discharged as follows: 82, 70, 146, 83, 126; mean, 101.4. The numbers employed were small and the results not quite so striking as in the first trial; but the difference between the means is significant, for a *t*-value of 2.52 was obtained. The value 2.31 appears in the tables of *t* for 8 degrees of freedom and a probability of 0.05. Hence, it appears that liver extract, like powdered dried liver,

serves as a source of vitamin G that does not promote the growth of the coccidia to the same extent as yeast.

Both series proved to be immune to reinfection. During 25 days on the experiment, the yeast recipients gained, on an average, 63 gm.; the liver extract recipients, 54.4 gm. While the gains of the latter were not quite so great as the expected, they were steady and amounted to 71 per cent of the original weight.

Third trial.—The diets were the same as in the first trial, except that the test diet was 6 per cent liver. Three test rats discharged 16, 11, and 31 million oöcysts; mean, 19.33 million. Three reference rats discharged 20, 52, and 50 million oöcysts; mean, 40.67 million. The numbers of hosts are small and by themselves would mean little; but the results correspond in general with the trend toward lower counts on the liver diet.

The mean increase in weight of the test series was 54 gm. over the first 18 days on the test diet; that for the reference series was 75 gm.

Fourth trial.—This experiment involved three series of rats: one, the control, on a diet consisting of the basal ration with 10 parts of the sugar substituted with Northwestern powdered yeast; another, the basal ration with 10 parts of the sugar substituted with powdered liver of our own manufacture similar to that used in the first trial; a third, the basal ration made up to 10 per cent with Wilson's liver meal, a commercial product. According to the packer's description, the latter product contained about 30 per cent livers dry weight, the balance being lungs and gullets. The organs were cooked under approximately 40 pounds pressure for 6 to 8 hours, and then subjected to pressing to remove as much grease as possible. Thus the commercial product differed from our product in both its constituents and method of manufacture. The rats were on the three diets for 9 days previous to the date of the first experimental infection.

Eight rats on the control diet each discharged millions of oöcysts as follows: 54, 90, 41, 72, 118, 40, 74, 19; mean, 63.5. Eight on the powdered liver prepared by us discharged as follows: 20, 24, 45, 73, 21, 29, 10, 28; mean, 31.25. The eight on Wilson's product: 15, 10, 4, 13, 18, 10, 37, 25; mean, 16.25. Without performing further statistical analysis, it is to be noted that the first series passed twice as many parasites as the second, and the second twice as many as the third.

During the firt 20 days on the experimental diets, the controls gained in mean weight from 106 gm. to 142 gm., or 36 gm. The recipients of our liver, from 102 gm. to 136 gm., or 34 gm. The lot on Wilson's liver meal increased in weight from 104 gm. to 111 gm. during the first 13 days, but were again back to 104 gm. by the twentieth day. It is evident that the liver meal lacked some growth factor, probably vitamin B. Our

liver and the powdered yeast supported normal growth. The experiment shows that it is possible to obtain normal growth and reduced parasite population; also, that the commercial all-animal product did not stimulate the parasite's growth.

Fifth trial.—This part of the experiment was intended to show whether the restricting action of liver was due to lack of a coccidium stimulant or to positive coccidistatic or coccidicidal properties. Six rats received a ration made up to 10 per cent with Fleischmann's powdered yeast, and the same number the same ration (yeast and all) with 10 per cent of the sugar replaced with the powdered liver of our own manufacture that we had shown to support a poor growth of coccidia in the host. The first series eliminated millions of oöcysts as follows: 164, 73, 180, 92, 610, 318; mean, 239.5. The second series: 104, 129, 196, 178, 186, 199; mean, 165.33. It is evident that the two series would have been practically the same had it not been for the one abnormally high count in the first. It is very seldom that such susceptible animals are found among rats infected with 1500 oöcysts each day for a number of days. This experiment shows that as much as 10 per cent liver in the ration containing 10 per cent powdered yeast does not exert a positive parasite restraining action on the numerical increase of the coccidia.

Sixth trial.—Five rats receiving the basal ration made up to 6 per cent with powdered yeast eliminated millions of oöcysts as follows: 139, 119, 123, 98, 53; mean, 106.4. Six on the same diet supplemented daily with 1 cc. liver extract equivalent to 4 gm. fresh liver: 73, 101, 90, 90, 66, 77; mean, 82.1. The first mean is somewhat higher than the second, but the difference is not statistically significant. The liver extract, then did not exert coccidium-restricting properties when yeast constituted part of the diet.

Seventh trial.—This experiment was planned with the end in view of testing the pathogenicity of the microörganism in relation to the diet rather than the numerical increase of the parasite in relation to the diet. Six rats averaging 84 grams in weight were placed on a diet consisting of beet sugar (57 pts.), non-extracted casein (18 pts.), Armour's commercial liver meal (10 pts.), cottonseed oil (5 pts.), cellulose flour (3 pts.), cod-liver oil (2 pts.), normal salt mixture (3 pts.), and rice polish (2 pts.). It may be noted that this is not an orthodox synthetic balanced ration, since the casein was the crude product. The rats made a mean gain of 58 gm. during the first 17 days on this diet. They were infected on the tenth, eleventh, and thirteenth days with 15,000 oöcysts of *E. nieschulzi* each, about 10 times as many as we ordinarily employ when it is desired to make studies on the numerical increase of the coccidium without producing pathogenic effects on the host. All of the rats lived, but one (with the highest oöcyst count) showed a definite diarrhoea on

the ninth day after infection. The oöcyst counts were as follows, in millions: 58, 341, 96, 390, 453, 288; mean, 271.

The reference series of 5 rats received the same diet as the test series except that the liver meal was omitted, and 10 parts of Fleischmann's powdered yeast substituted. The average gain during the first 17 days was 66 gm. The infective doses of the microorganism were the same. All 5 rats lived, but four of them developed a severe diarrhoea on the eighth and ninth days. The one which did not suffer passed the smallest number of oöcysts. The oöcyst counts were as follows: 463, 141, 438, 517, 629; mean, 437.

The number of animals employed in this experiment was small, but it is indicated that when the number of oöcysts appearing in the feces is reduced through the diet, the clinical symptoms are likewise less severe.

SKIM-MILK AS A SOURCE OF VITAMIN G

These experiments were carried out only on rats. The parasite was *Eimeria nieschulzi* (*E. miyairii*). The rats were kept on the experimental diets for 10 days previous to the first experimental infection with 1500 sporulated oöcysts, and infection was repeated the next and third day after the first infection.

First trial.—Seven rats were put onto a diet consisting of the basal ration made up to 7 per cent with powdered skim-milk and 1.5 per cent with rice polish. During the first 20 days, these rats made a mean gain of 28 gm. Seven rats received the basal ration made up to 7 per cent with powdered skim-milk, 6 per cent with Fleischmann's powdered yeast, and 1.5 per cent with rice polish. They made a mean gain of 47 gm. during the first 20 days. The first series eliminated millions of oöcysts individually as follows: 2, 26, 56, 11, 44, 16, 18; mean, 24.71. The second series: 89, 86, 22, 23, 90, 113, 39; mean 66. In addition, there were 4 rats that received the same ration as the first series except that there was 1.0 per cent ammonium sulphate. These eliminated millions of oöcysts as follows: 44, 50, 4, 48; mean, 34. Their mean weight gain was only 4 gm.

Second trial.—Four rats receiving the basal ration made up to 6 per cent with Fleischmann's powdered yeast eliminated millions of oöcysts as follows: 172, 176, 175, 165; mean, 172. Their mean gain for the first 21 days was 80 gm. Three receiving the same diet except that the yeast was increased to ten per cent: 220, 226, 177; mean, 207.67. Their mean weight increase was 69 gm. Four rats receiving the basal ration made up to 7 per cent with powdered skim-milk eliminated millions of oöcysts as follows: 8, 2, 17, 5; mean, 8. The mean gain was 19 gm. Four others receiving the basal ration made up to 6 per cent with rice polish

eliminated as follows: 49, 29, 156, 38; mean, 68. Their mean weight increase was 34 gm.

From these experiments it may safely be concluded that skim-milk of itself as a source of vitamin G offers considerably less growth stimulus to *Eimeria nieschulzi* than powdered yeast, or powdered yeast supplemented with skim-milk. Rice polish seems to favor the increase of the coccidium much less than yeast, but considerably more than the skim-milk.

DISCUSSION

It is believed that the foregoing controlled experiments have definitely proved that significant reduction in numbers of oöcysts eliminated by an experimentally infected host can be effected through manipulation of materials incorporated into the dietary formula to supply vitamin G (and sometimes B also), and that such parasite reduction can be attained in a young host experiencing normal growth. Former experiments by the writers had shown that diets lacking vitamin G, or inadequate in respect to this vitamin, do not favor coccidian infections of normal intensity; and, of course, adversely affect the growth and development of the host. At first vitamin G in the orthodox sense (a complex though it is) was believed to be the coccidium stimulant, on account of its thermo-stable property. The newer experiments with liver and skim-milk products, however, reveal that it is possible to provide quantities of vitamin G adequate for the usual or normal host growth, and coincidently restrict the numerical increase of the coccidium to far below normal. These are the facts, but what mechanism operated towards the attainment of the restricted parasite development? Conceivably, there were a number of possibilities that will be considered one by one.

When the writers first reported the parasite-restriction phenomenon in hosts on vitamin G deficient diets, it was suggested to them orally that the parasite's growth might be a function of that of the host. This did not seem likely at the time; for rats suffering from vitamin B deficiency and receiving a ration with 10 per cent of autoclaved yeast as a source of vitamin G made less growth, as compared with the controls, than rats receiving wheat germ extract as a source of vitamin B and no intentionally supplied source of vitamin G, and still harbored "normal" infections, while the vitamin G deficient series was experiencing infections only a third or fourth as heavy as the controls (6). The demonstration of simultaneous normal host-growth and restricted infection would likewise seem to remove the necessity for further consideration of the growth function hypothesis.

The possibility that the "non-absorbable fraction" (*i.e.*, non-flavin or P-P) in liver and skim-milk is qualitatively different from that in yeast, rice polish, wheat germ, and other plant products must also be con-

sidered, with little likelihood of reaching a definite conclusion. At the present time, the vitamin G complex is being actively investigated in many laboratories. It is almost safe to say (after the reading of much literature) that the physiological roles of certain parts of the complex are but hazily known, to say nothing of chemical structure. The flavin or absorbable fraction occurs in liver, skim-milk, and yeast, all three, so it is not under consideration as affecting the coccidia differentially according to source.

Very serious consideration was given to the discovery by Faust and his collaborators (10, 11) of "the amebostatic and possibly amebacidal properties of whole raw liver and of powdered liver extract administered orally to dogs which had been experimentally infected with human strains of *Endamoeba histolytica.*" Furthermore, their studies showed that liver favored healing of the denuded intestinal wall, appeared to prevent to a measurable degree invasion by bacteria, and perhaps neutralized the toxic effect of protein-split products resulting from tissue degeneration. It is not unlikely that attenuated symptoms manifested by chicks on the liver diet (13) were in some degree due to these latter beneficial properties of liver, as well as to its erythropoietic function. But how much of the improved physical state was due to these direct effects and how much to reduced parasite development is difficult to evaluate. At any rate it seems certain that liver and skim-milk powder do not exert a pronounced coccidistatic or coccidicidal action on coccidia, as was ascertained by comparing oöcyst counts in rats receiving liver (or skim-milk) as the sole source of vitamin G and rats receiving both liver (or skim-milk and yeast as sources of vitamin G. If there was a direct adverse effect of the liver or skim-milk on the protozoön, it would have been revealed by this test, unless the yeast exerted an overpowering growth-stimulating action. Since the usual parasite development occurred when the proportion of yeast was as low as 6 per cent, it seems likely that nothing of the sort operated to nullify possible positive antagonistic action of liver and skim-milk powder toward the microörganism.

Then there remains the possibility that the reaction of the intestine is modified by the parasite-restricting diet, and that the altered reaction is unfavorable to the coccidium. One of us (N. F. M.) has made some preliminary hydrogen-ion determinations on rats that showed light coccidium infections owing to a diet deficient in vitamins B and G. No change from the normal was observed. The reaction of the intestine of rats on the particular liver- and skim-milk-containing diets used in this experiment remains to be determined. A previous study (4) gave no indication of a change of pH in the intestines of rats receiving the growing ration made up to 40 per cent with powdered skim milk.

The picture as a whole suggests that there occurs in powdered yeast,

wheat germ, rice polish, and other plant products a certain coccidium-stimulating substance. It is most likely to be detected in connection with vitamins G and B, and it is thermostable. It is present in scanty amounts in liver and skim-milk powder, both excellent sources of vitamin G. Rice polish may be used in small amounts to supply vitamin B, which is not plentiful in liver and skim-milk, in synthetic diets employing the latter materials as sources of vitamin G. Rice polish, however, contains appreciable amounts of the coccidium-accelerant, and must be used sparingly. The assumption of a definite coccidium-growth-promoting factor is strengthened by a deductive analysis of the following train of events: First, it was discovered that coccidian development is greatly restricted by leaving vitamins B and G out of the diet (3). Then it was learned that the development was still restricted when vitamin B was supplied and vitamin G left out (6). The next step consisted in achieving more and more host growth as more and more liver and liver extract was added to the diet, with the parasite's development still restricted (7).

Finally, by adding adequate amounts of powdered liver and rice polish to the basal diet, both normal growth of host and parasite reduction were effected. It seems entirely justifiable from the very nature of the circumstances to make the tentative deduction that the parasite-restricting effect is due to the partial absence from the diet of a peculiar nutrient of the coccidium. The rat and chicken (13) appear to be able to grow with this nutrient at a low level in the diet, at least for a period sufficient in length to demonstrate the inhibiting effects on the coccidium.

Or, to put it positively, there occur in many materials used as food for animals substances that are capable of stimulating the multiplication of coccidia in their hosts. A comparison may perhaps be made with bios, the yeast stimulant. Whether this hypothetical material acts directly upon the coccidium as it develops in the interior of the host cell, or indirectly by affecting the metabolism of the host cell, cannot even be conjectured at the present time. Such a hypothesis would serve as a fitting working basis in future biological assays of foods for their coccidium-promoting properties. Its ultimate validity, of course, would depend upon the isolation of the stimulant. For convenience of terminology we are suggesting the name coccidibios for this hypothetical coccidium stimulant. We venture to suggest that the attack upon the diseases called collectively coccidiosis can best be made by studying foods with respect to their coccidium stimulation, as well as their suitability for the host. It may well be that other parasites are stimulated by the same or other nutrients in foods. The senior author's observation (12) that *Hymenolepis nana* does not lay eggs when the rat host is kept on an exclusive diet of milk is a case in point.

SUMMARY

In experiments on rats raised in the laboratory and known to have been free from accidental infection up to the date of experimental infection with *Eimeria miyairii,* it has been shown that diet bears a very close relationship to the severity of coccidian infection. The criterion was in most cases the numbers of oöcysts eliminated. Yeast, when used in the ration as the source of vitamin G, stimulated the production of oöcysts to the apparent maximum. When powdered liver or a commercial liver meal were used as a source of vitamin G, the production of oöcysts in the infected host was much reduced. Skim-milk powder appears to contain even less of the coccidium stimulant than liver. Rice polish contains much less of it than yeast, but more than powdered liver. Evidence is produced for the belief that the reduced oöcyst counts are actually due to the absence of something from the diet, rather than to the presence of a positive arrestor of the parasite's development. The name coccidibios is suggested for this newly discovered factor in food which favors the development of the coccidium *Eimeria nieschulzi* in the rat.

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THE LARVAL STAGES OF *WOHLFAHRTIA VIGIL* (WALKER)

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Although the larval stages of *Wohlfahrtia vigil* (Walker) have been known for many years they have never been described in sufficient detail for a satisfactory determination of the species without rearing the adults. The descriptive notes by the writer (1919, 1922) and the description of the first instar by Johannsen (1921) are not complete enough for accurate diagnosis, particularly in the case of the third instar, which was made from scanty and poorly preserved material. The numerous rearings of this species by Dr. Norma Ford, who has made an extensive study of its behavior, have provided ample material for description and, in view of the importance of the species both from the medical and veterinary standpoints, the following descriptions have been prepared in order to present definite characters by which each larval instar may be recognized.

First Instar: (figs. 1, 2). Length 1.5 mm. at time of hatching to 3.5 mm. at time of first moult. Body when moderately extended, 4.25 to 4.5 times as long as the greatest breadth, which is in the region of segments 7–9. Segments 1 and 2 relatively longer than in the later instars (*cf.* figs. 2 and 8). Seg. 1 (pseudocephalon) broadly rounded or subtruncate in front: antenniform appendages (fig. 3) much more conspicuous than in later instars, consisting of a broad base bearing a minute lateral tubercle and an elongate-conical distal segment, tapering to a bluntly pointed apex: "maxillary palps" (fig. 4) in the form of small rounded discs bearing an irregular group of sensillae of several sizes: ventral surface longer than dorsal, with three delicate oral grooves.

Segs. 2–5 with complete spinous annuli, the spines distributed singly in transverse rows, alternating in position in successive rows, stoutest on 2, (fig. 5) more slender on the remaining segments (figs. 6, 7) and somewhat shorter on the last two or three (fig. 7). On the first two segments the larger spines measure about 8 to 8.5 μ in length.

Anterior spinous areas (figs. 1, 2, a2–a9) present on segs. 2–12, but complete annuli only on 2–9, becoming confined on 10, 11, and 12 to the ventral surface. The anterior spinous areas on 2–5 are simple belts without bare ventral areas, while the corresponding areas on 6–11 are each divided on the ventral surface into two transverse strips enclosing a bare area, which covers the greater part of a row of three nearly contiguous prominences. These prominences are very slight in the first instar and are indicated on fig. 2 only by the positions of the bare areas

(*cf.* fig. 8). On seg. 12 only the median prominence is distinct at this stage, while the anterior spinous area is represented only by a ventral transverse strip. These ventral prominences lie in the same transverse plane as the lateral fusiform pads (fp) which are well marked and covered with small spines. They appear as though intersegmental in position, being situated on each side just in front of the anterior margins of 6–11 and there is a trace of a pair in front of 12. There are 9 or 10 rows of dorsal spines in the anterior spinous area of seg. 2, 6 or 7 rows on segs. 3 and 4, and 5 or 6 rows on segs. 4–8, but only 3 rows on seg. 9.

The posterior spinous areas appear on segs. 5–11, increasing in extent posteriorly from a very small ventral strip of 3 rows on seg. 5 to a complete though narrow annulus on 8 and successively wider annuli on 9–11. The smooth areas between the spinous areas are marked by delicate folds but there are as yet none of the tubercles which appear later, except the six very minute ones surrounding the spiracular pit, of which four are above the pit and two below.

Posterior spiracles (fig. 18) separated by a space narrower than the transverse diameter of a single spiracle; each spiracle about as broad as long, the peritreme undeveloped ventrally, with a single orifice, which is bifid in its upper half, protected by slender hairlike processes.

Cephalopharyngeal sclerites: Median or labral hook (fig. 12, 13 mh) very large, strongly arched near the stout base, with a long tapering apex. Lateral hooks[1] (fig. 12, 13 lh) small, slender and straight, their tips more or less decurved, their bases articulated with a pair of stouter sclerites, nearly vertically placed, one on each side of the base of the median hook. Articulating with the posterior bifurcation of the base of the median hook are the two straight rod-like parastomal sclerites (ps) which overlie the rudiments of the hypostomal sclerites (hs) and are connected behind with the ventral prolongations of the pharyngeal sclerites. The latter are comparatively narrow and are inclined slightly backwards. The dorsal arch (fig. 12) is incomplete, consisting of rather short, slender, horizontal processes, which converge at an acute angle but do not quite meet. The dorsal cornua (dc) are long and slender, their length increasing with growth. The ventral cornua (vc) are somewhat shorter and less sclerotized at first, but rapidly increase in length and soon become as long as the dorsal cornua.

The first moult usually occurs during the second day after birth.

Second Instar: (fig. 8). Length 3.5 to 6.75 mm. Similar in form to the first instar, except in the relatively smaller size of the first two segments. First segment truncate or slightly emarginate in front, the antenniform appendages greatly reduced (fig. 9) : ventral surface with numer-

[1] These are the structures which have been termed "oral rods" (v. Walker '22).

ous oral grooves: oral aperture bounded behind by a bluntly pointed lobe: lateral mouth-hooks (figs. 14, 15) much larger than in stage 1 with decurved tapering apices. Anterior spiracles (fig. 19, a sp) present in the form of small rounded lobes, each bearing a row of nine minute circular openings, scarcely elevated upon papillae.

The spinous belt of seg. 2 (fig. 8, a2) is relatively small, bearing numerous but, for the most part, unpigmented cuticular processes, scarcely firm enough to be called spines. The spinous belts on segs. 3–5 (fig. 8) are simple, as in stage 1. The spines on these and the following segments do not diminish in size appreciably on the posterior segments as they do in stage 1 and are relatively shorter and less tapering than in that stage, occasionally bifid (fig. 11). They are arranged in short series, usually 2 to 4, separated by intervals, and these series form irregular transverse rows (fig. 17). The larger spines measure about 9 or 10 μ in length.

The anterior spinous areas are complete annuli as far back as seg. 9 or 10, posterior to which they are represented only on the ventral surface. Each ventral area from seg. 6 to 12 is divided into two strips (fig. 8) the intervening bare area being completely enclosed, except on seg. 12, on which the two strips are separated. The three ventral prominences present on each of these segments, are partly covered by the anterior strip but otherwise occupy the bare areas.

The posterior spinous areas are represented on segs. 5 and 6 by a narrow ventral isolated strip of about three rows of spines, (fig. 8, p5, p6) immediately in front of the anterior spinous area of the succeeding segment. The strip is a little longer on seg. 6 than on 5, while on the remaining segments the strips become complete annuli, increasing in width posteriorly, being composed of four rows of spines on 7 up to about six rows on seg. 11. The lateral fusiform pads (fp) occupy the intervals between the posterior spinous belt of one segment and the anterior belt of the next segment, where adjacent belts are complete. What is evidently the representative of the lateral fusiform pad of seg. 12 is merged in the posterior spinous belt of seg. 11.

Alternating with the spinous annuli are transverse series of small tubercles, which are arranged as in the third instar, in which they are much more distinct. They will therefore be considered in the description of the third instar.

The posterior spiracular pit is deeper than in the first instar and is encircled by tubercles as in the third instar, though they are very minute.

The posterior spiracles (fig. 19) are separated by a space which is narrower than the diameter of a single spiracle. They are broadly rounded, their dorso-ventral diameter about a third longer than their transverse diameter, the peritreme incomplete ventro-mesially. There are

now two spiracular slits in each plate, the outer slit a little longer than the inner one, its lower half bent mesially. The inner slits are straight and slightly inclined dorsally towards the middle line. Each slit has a thickened rim with a very uneven inner margin from which about 8 or 9 cuticular strands divide the slit irregularly into pores of various sizes.

Cephalopharnygeal sclerites. These have been already described (Walker, '22) but examination of further material shows certain differences which suggest that they are somewhat variable. The lateral mouthhooks arise each from a thick base with a prominent dorsal angle and a blunt ventral process. The hooks of the specimen here figured (fig. 15) are stouter and less tapering than in the one figured in the paper just cited. The rudiment of the dental sclerite is seen just behind the ventral process. The hypostomal sclerite is H-shaped as viewed dorsally. In this specimen, unlike the one figured in the earlier paper, it is apparently not separate from the pharyngeal sclerite and the ventral prominence is much less marked. The parastomal sclerites now have the form of two slender rods projecting forward from the pharyngeal sclerites over the hypostomal sclerite. The pharyngeal sclerites resemble more closely those of stage III, as indicated by the broader lateral plates and the bifid and more heavily sclerotized cornua, both dorsal and ventral.

The most interesting difference between the first and second instars is the great reduction in the size and firmness of the spines of the second or prothoracic segment and, to a less degree, those of the third segment. These spines are no doubt an important adjunct to this large labral hook in enabling the first stage larvae to penetrate the skin of the host, a function that is not required of them after the larva has entered the host tissues.

The second moult takes place on the third day after birth.

Third Instar: (fig. 22–24). Length 7 to 16.5 mm. Differs in form from the second instar, and still more from the first in the relatively smaller size of the anterior segments, the pseudocephalon being much smaller, as is likewise true in lesser degree of the next four or five segments. The body is broadest at about the 8th segment, tapering cephalad and narrowing slightly caudad to the base of seg. 11, then more abruptly to the posterior spiracular pit. The pseudocephalon is distinctly bilobed in front, the obtusangulate lobes bearing the antenniform appendages at their apices.

Spinous annuli similar to those of seg. 2, except that, for the most part, they are broader and are occupied by a considerably larger number of rows of spines, which are relatively much smaller than in stage 2 (largest spines about 15 to 16 μ in length). Their form and arrangement in short series is similar, but the series are arranged still more irregularly in transverse and oblique, more or less undulatory rows. The spines of

seg. 2 are very feeble, though numerous on the ventral surface, and largely unpigmented. Segments 2 to 5 have anterior spinous annuli only, seg. 6 has an anterior annulus and a narrow posterior ventral strip: 7 to 11 have complete anterior and posterior annuli, while 12 has a ventral anterior spinous area but is without spines dorsally. A very narrow strip of about three rows of spines at the base of the anal segment possibly represents the posterior spinous area of seg. 12. Viewed from above the anterior spinous areas are well developed as far back as seg. 10, though somewhat narrower on this segment than on seg. 9. On seg. 11, however, it is reduced to a few rows of spines. The posterior annuli, on the other hand, begin on seg. 7 with a narrow strip, increase in width up to 10 or 11. Viewed ventrally the chief differences from stage 2 are the greater width of the spinous annuli, the development of the anterior spinous areas of segs. 10 and 11 into complete or, in the case of 11, almost complete annuli, and the much greater number and relatively smaller size of the spines. The lateral fusiform pads and the median and submedian prominences are larger and more conspicuous than in stage 2.

The tubercles which are arranged in transverse series alternating with the spinous annuli are also more prominent and clearly defined than in the second instar. They are arranged as follows: Each segment from 3 to 12, inclusive, is encircled by a ring of tubercles, which, anterior to seg. 10, occupy the anterior half of each segment, while those of 10 and 11 are near the middle of their respective segments, and those of 12 surround the margin of the posterior spiracular pit. The tubercles of segs. 3 to 5 are crowded and ill-defined, while those of the remaining segments, except the last, may be divided into dorsal lateral and ventral groups.

The dorsal group consists of (a) six small tubercles (fig. 22, d), three on each side of the median line, which are close together on the more anterior segments but spread apart posteriorly, especially on segments 10 and 11: and (b) a larger tubercle on each side of the median group and separated from it by a distinct interval.

The lateral group consists of two tubercles, a smaller dorso-lateral (dl) and a larger lateral (l), in alignment with the dorsal series: and two other small tubercles which are displaced from this series. These form with the dorso-lateral tubercles an oblique row running downwards and forwards.

The ventral group (v) is in alignment with the main series of dorsal and lateral tubercles and consists usually of two transversely elongated and often contiguous tubercles, separated mesially by a considerable interval. These tubercles vary considerably in form and sometimes one of them is subdivided. Anterior to this series of ventral tubercles there

is a transversely elongated median fold with a very small tubercle on each side in the same transverse plane.

The anterior spiracles are broadly arcuate, each bearing 9 spiracular papillae.

The posterior spiracular pit (fig. 21) is deeper than in preceding stages, broadly crescentic in outline, the roof being concave and the floor convex. The pit is surrounded by a thickened ridge bearing six pairs of tubercles, three above and three below. Of the three upper pairs the outermost is the largest, the inner or median pair next in size, while the intermediate pair is smallest. The lower tubercles are all very small.

The posterior spiracles (fig. 20) are at first close together but when growth is completed they are separated by a space about equal to the diameter of one of the plates. The peritreme of each plate, which is incomplete ventrally, is nearly straight on the mesial side, but is strongly curved or bent laterally. The outermost of the three slits is slightly shorter than the other two, which are about equal in length: the innermost slits of the two plates are slightly convergent dorsally, the middle slits parallel and the outer slits slightly divergent. In the details of their structure they are similar to those described for stage 2. Below the ventral gap in the rim is a slight vestige of a "button."

The cephalopharyngeal sclerites (fig. 16) have already been described (Walker, '22) but are refigured here for the sake of completeness. The only differences from those of the second stage (fig. 15) are the relatively shorter and much deeper bases of the lateral mouth hooks, which are not concave.

Since the length of the larval period, according to Ford ('36), at optimum temperatures is seven to nine days, the third stage occupies four to six days.

The third stage larva of *Wohlfahrtia vigil* resembles that of *W. magnifica* in its general characteristics as judged by the figures and brief description of the latter by Patton and Evans (1929, 449–450). The spinous areas, as far as can be seen in the figure, are similarly distributed but the spines are evidently much coarser. The anterior spiracle differs in having only five or six papillae instead of nine or ten as in *W. vigil*. The peritreme of the posterior spiracles appears to be stouter in *W. magnifica*. Differential characters for second stage larvae of these two species were given by the writer (Walker, 1920). The larva of *W. magnifica* was first described by Portchinsky (1875).

The larva of *Wohlfahrtia* sp. described by Sinton ('21) resembles *W. magnifica* in having only five papillae on the anterior spiracles. The peritreme of the posterior spiracles is likewise heavier than in *W. vigil* and its ventro-mesial extremities are bifid. The arrangement of spinous areas, as shown in Sinton's figure (*loc cit.*, fig. 7), is very complex and

peculiar and considerably different from that of *W. vigil*. The tubercles surrounding the posterior spiracular pit appear to be larger than in either of the other species.

Whether any generic characters exist for the larvae of *Wohlfahrtia* cannot yet be stated. Root ('23) describes two distinct types of posterior spiracles in the genus Sarcophaga based on a comparison of nine species.[2] To quote from this writer: "In the more common type . . . the inner portion of the ring is nearly vertical and ends in a prominent knob, while the slits are slender and are crossed by a larger number of anastomosing bars. In the other type . . . the inner portion of the ring curves outwards and tapers to a point, ending in contact with the lower end of one of the three slits, and the slits themselves are broader than in the first type and are crossed by a much smaller number of distinct bars." In the form of the slits and the number of bars *Wohlfahrtia* resembles the second type but the rim resembles that of the first type except in the lack of a knob.

In the species of Sarcophaga studied by Metcalf the branches of the anterior spiracles varied from 10–13 in *S. securifera* Villineuve to 30–35 in an undetermined species (*Sarcophaga* "F"). In the species of *Wohlfahrtia* whose larvae are known the branches of the anterior spiracles vary from 5 to 9 (rarely 10). This character may thus approximate a generic one, but, in view of the large number of species, particularly of Sarcophaga, whose larvae are unknown, it is impossible to employ it otherwise than in the separation of species.

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[2] *S. communis* Parker and *S. communis* var. *ochracea* Ald. are here considered as separate species since their posterior spiracles belong to opposite types.

Explanation of Plates

Plate I

Wohlfahrtia vigil (Walk.) 1–7, first instar: 1, dorsal view: 2, ventral view: 3, antenniform process: 4, "maxillary palpus": 5, spines from second or prothoracic segment (a2): 6, spines from fourth segment: 7, spines from tenth segment: 8–10, second instar: 8, ventral view: 9, antenniform process: 10, spines from third segment: 11, third instar, spines from third segment, showing arrangement in broken transverse rows.

a2, a3 . . . a9, anterior spinous areas: *p7 . . . p11,* posterior spinous areas: *fp,* fusiform pad.

Plate II

Wohlfahrtia vigil (Walk.) 12–16, cephalopharyngeal sclerites: 12, first instar, dorsal view: 13, first instar, lateral view: 14, second instar, dorsal view: 15, second instar, lateral view: 16, third instar, lateral view: 17, second instar, spines from third segment, showing arrangement in transverse groups.

mh, median hook: *lh,* lateral hook: *ds,* dentate sclerite: *hs,* hypostomal sclerite: *ps,* parastomal sclerite: *ph,* pharyngeal sclerite: *da,* dorsal arch: *dc,* dorsal cornua: *vc,* ventral cornua: *asp,* anterior spiracle.

Plate III

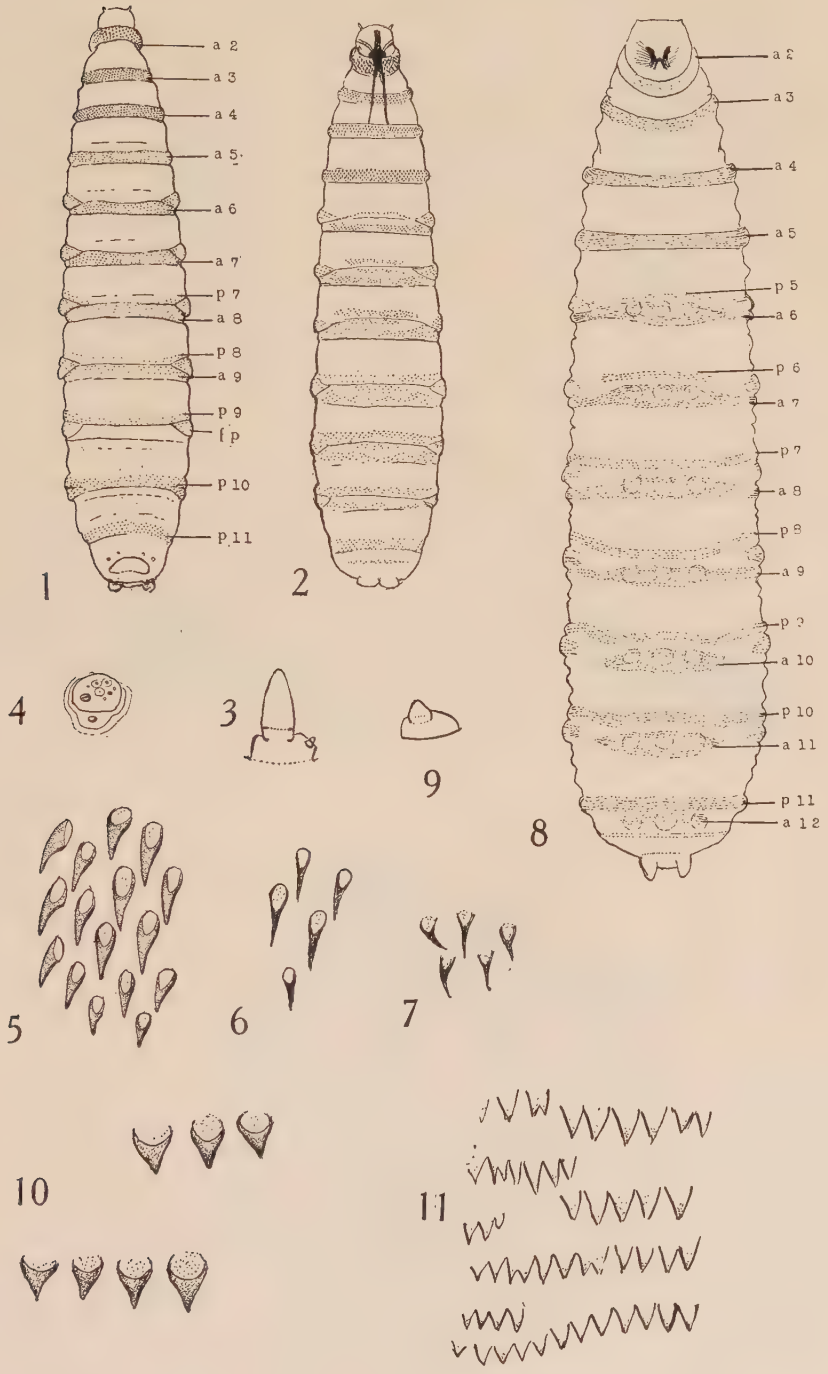
Wohlfahrtia vigil (Walk.), posterior spiracles: 18, first instar: 19, second instar: 20, third instar: 21, view of posterior end of third instar, showing spiracular pit and tubercles.

Plate IV

Wohlfahrtia vigil (Walk.), third instar: 22, dorsal view: 23, ventral view: 24, lateral view.

d, dorsal tubercle: *dl,* dorso-lateral tubercle: *l,* lateral tubercle: *v,* ventral tubercle. Other lettering as before.

PLATE I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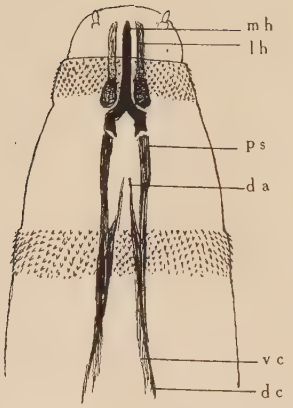

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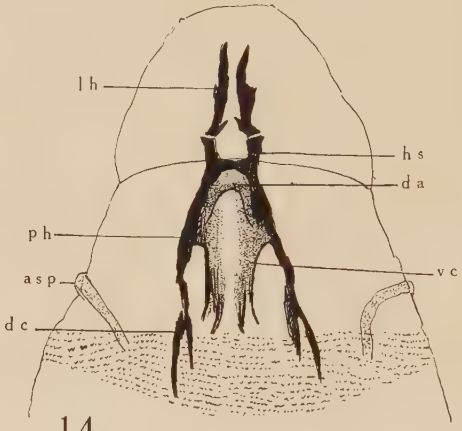

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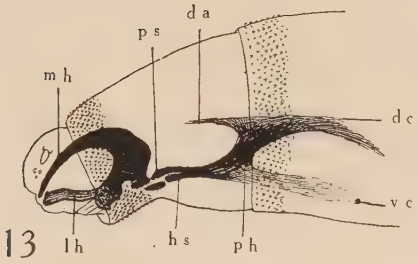

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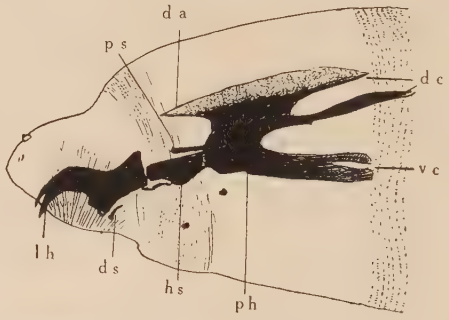

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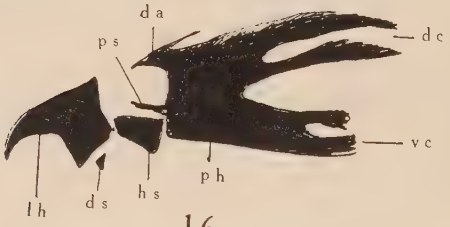

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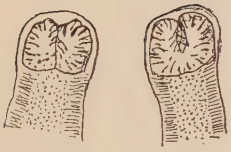
PLATE III



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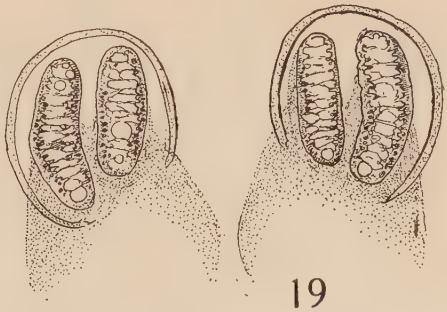

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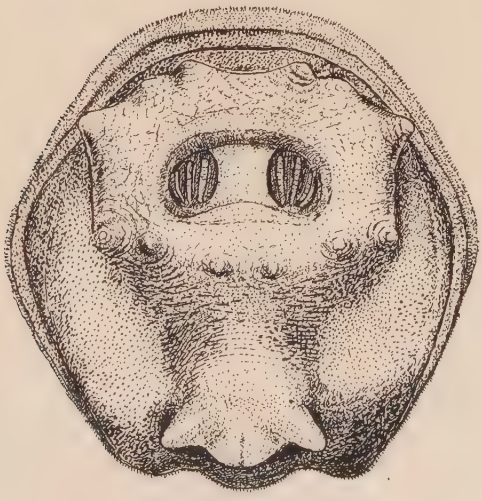

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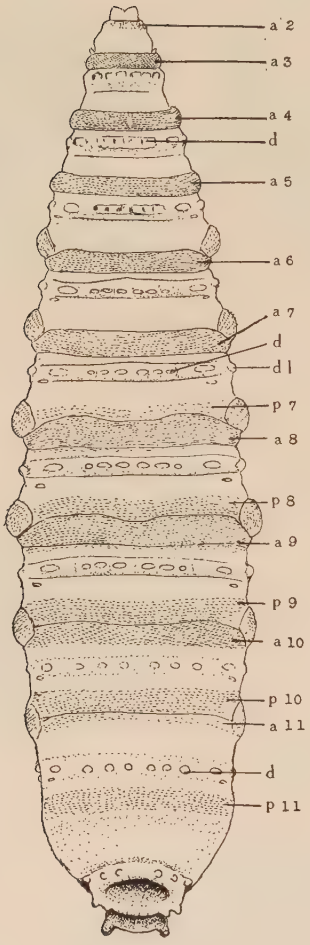
PLATE IV



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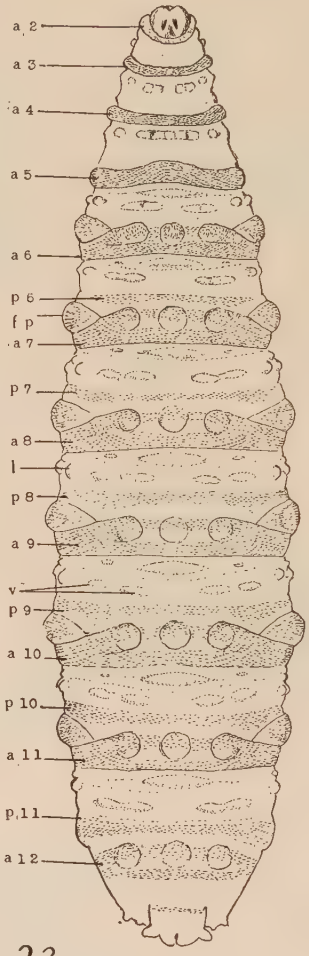

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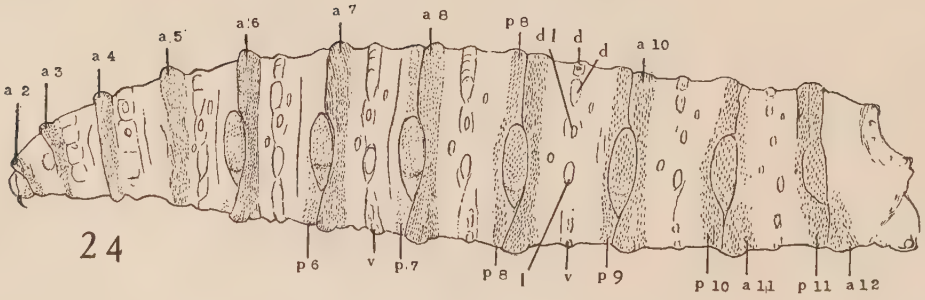

24

THE EFFECTS OF EXPOSURE TO DIFFERENT DEGREES OF TEMPERATURE ON THE ETIOLOGICAL AGENTS OF BOVINE ANAPLASMOSIS AND PIROPLASMOSIS

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The experiments herein reported were undertaken to determine whether cattle could be immunized with the etiological agent of anaplasmosis, *Anaplasma marginale,* that had been killed *in vitro* by heating or freezing the red blood cells in which this agent was present on the assumption that death would occur at 60° C. and also at −78.6° C., the latter being the temperature of "dry ice." However, since experiments 1 and 2 of the present paper did not support this assumption, the succeeding experiments were undertaken to determine the thermal death point of *Anaplasma* more precisely and also to learn to what extent the virulence of this organism could be reduced by heating the infected red blood cells to a temperature slightly below the thermal death point. Another objective was to compare the thermal death point of the causative agent of anaplasmosis with that of *Babesia argentina* and of *B. bigemina;* the two latter organisms are definitely known to be endoglobular protozoan parasites, while the precise biological status of *Anaplasma marginale* is still open to question.

EXPERIMENTAL PROCEDURE

Since the etiological agent of anaplasmosis occurs in the red blood cells, these cells only were used for the purpose of determining the thermal death point; the cells were separated from the serum of defibrinated blood, washed in physiologic saline and concentrated by centrifugation. Some leucocytes and thrombocytes were probably mixed with the erythrocytes that were subjected to the various heating tests described in this paper. These concentrated cells were heated in a water bath. When the quantity used was greater than 20 cc. the cells were transferred to a 250 cc. Ehrlenmeyer flask which was held in the bath by hand and gently rotated throughout the heating period. Care was taken to keep the flask immersed well below the surface of the cells and to avoid splashing on the sides of the flask above the water line. When the quantity was 20 cc. or less the cells were transferred to ordinary thin-walled test tubes, the latter being held in the bath in a wire basket. At the beginning of each immersion period the temperature of the cells was that of the room. Except in experiments 2 and 9, the temperature of the

cells at the end of the heating period was not determined. The concentrated cells were injected intravenously into the cattle.

EXPERIMENTAL DATA

Experiment 1

On April 22, 1935, 150 cc. of red blood cells from bull 123, a clinical splenectomized case of anaplasmosis, with 20 anaplasmata per 1000 red cells, were packed in a flask in finely crushed "dry ice" and placed over night in an electric refrigerator. At the end of 18 hours the flask was still completely packed in the "ice" and was broken, and the cells were frozen into a solid mass. After thawing at room temperature the cells were found to be completely laked, and this material was then injected into heifer 131. On May 22, 30 days after injection, this heifer developed a severe case of anaplasmosis; the red cell count dropped to 2,200,000 per cubic millimeter of blood.

This experiment showed that exposure for 18 hours to the temperature of dry ice failed to kill the causative agent of anaplasmosis.

Experiment 2

On April 16, 100 cc. of red blood cells from bull 123 were heated 20 minutes at 60° C.; at the end of this period the temperature of the cells was only 56° C. The temperature of the bath was then raised to 64° C. for 5 minutes and at the end of this period the temperature of the cells was 60° C. The heated cells were injected into heifer 127. At 60° C. the color of the red blood cells changed from bright red to dark red and their consistence from that of a uniform suspension to a viscous mass. On April 19 in an attempt to heat to 60° C. without producing these changes, 75 cc. of red blood cells from bull 123 were heated in water bath at 62° C. In 25 minutes the temperature of the cells reached 60° C. and they were immediately taken out of the bath. Some changes in color and consistence of the cells were noted but these were not so marked as in those heated previously. A stained smear from this sample showed that the red blood cells were swollen and of uneven outline, but the anaplasms stained normally. These heated cells also were injected into heifer 127. On May 22, 33 days after the second injection, anaplasms were found on microscopical examination of the blood of this heifer but at no time did the animal show clinical symptoms of anaplasmosis. A count made on May 25 showed 6,160,000 red blood cells per cubic millimeter of blood, and a Wintrobe hematocrit reading showed 39 volumes per cent of these cells. Previous examinations had shown up to 7 million red blood cells per cubic millimeter and hematocrit readings of 42 to 46 volumes per cent.

The data obtained in this experiment showed that heating to 60° C.

for a period less than 5 minutes failed to kill the causative agent of anaplasmosis but greatly reduced its virulence.

Experiment 3

Heated cells. On May 28, 150 cc. of red blood cells from heifer 131 which, as noted in experiment 1, developed anaplasmosis on May 22, were heated 1 hour at 60° C. and then injected into bull 134. Anaplasmosis did not develop as a result of this injection.

Unheated cells. On July 16, 50 cc. of unheated defibrinated blood from heifer 131 were injected into bull 134. On August 3, eighteen days following the injection, this bull showed clinical symptoms of anaplasmosis and death occurred on August 9.

This experiment showed that exposure for 1 hour at 60° C. killed the causative agent of anaplasmosis, and that injection of the killed agent into a susceptible bull did not render him immune to the disease when subsequently infected with virulent blood.

Experiment 4

On August 9, 20 cc. of red blood cells from bull 132, a case of anaplasmosis diagnosed on August 3, were heated 20 minutes at 60° C. and injected into bull 135. This bull did not become infected, although in the next experiment (experiment 5) he proved to be susceptible to anaplasmosis, thus demonstrating that exposure for 20 minutes to 60° C. killed the causative agent of anaplasmosis.

Experiment 5

Unheated cells. On August 9, 25 cc. of unheated red blood cells from bull 130, a case of mixed infection with *Babesia bigemina* and *Anaplasma,* diagnosed 30 and 214 days previously, were injected into bull 136. On August 15, this bull showed evidence of *Babesia bigemina* infection and on September 3 he was found to be infected with *Anaplasma.*

Heated cells. On August 9, 25 cc. of red blood cells from bull 130 were heated for 15 minutes at 60° C. and injected into bull 137, a susceptible bovine over 6 years old. This bull did not develop piroplasmosis or anaplasmosis; therefore, on September 16, 20 cc. of red blood cells from bull 136 were heated 10 minutes at 57° C. and 10 cc. were injected into bull 137 and 10 cc. into bull 135, the latter bull having failed to react in experiment 4. Neither bull developed piroplasmosis nor anaplasmosis. On October 22, the same bulls were again injected each with 10 cc. of red blood cells from bull 136, the cells having been heated for 10 minutes at 54° C. Since again no symptoms developed in either bull on November 29 they were each injected with 10 cc. of red blood cells from bull 136, the cells having been heated for 10 minutes at 48° C. Neither

piroplasmosis nor anaplasmosis was transmitted. On January 15, 1936, 10 cc. of red blood cells from bull 144, a case of anaplasmosis diagnosed 20 days previously, were heated 10 minutes at 48° C. and injected into bull 137. On February 17, 33 days later, this bull reacted to anaplasmosis and died on February 23. On January 20, bull 135 was splenectomized because non-clinical anaplasmosis was suspected; however, no post-splenectomy symptoms of anaplasmosis occurred and anaplasms could not be found in the blood smears. On February 6, 10 cc. of red blood cells from steer 144, a case of anaplasmosis as noted above, were heated 15 minutes at 48° C. and injected into bull 135. On March 6, 29 days later, this bull developed clinical anaplasmosis and died on March 10.

The above data show that exposure for 15 minutes at 60° C., and for 10 minutes at 57° C., 54° C., and 48° C., respectively, killed the causative agents of both piroplasmosis and anaplasmosis, the above agents occurring in mixed infections; that in a pure infection the latter agent survived exposure for 10 minutes at 48° C.; and that four injections of red blood cells containing the killed agent of anaplasmosis into each of 2 bulls failed to produce immunity to anaplasmosis.

Experiment 6

On September 30, 1935, 10 cc. of red blood cells from bull 130, a mixed case of *Babesia bigemina* and *Anaplasma* infection, as noted in experiment 5, were heated 10 minutes at 54° C. and injected into each of 2 bulls, Nos. 140 and 141; both were susceptible animals over 6 years old. Neither disease was transmitted. On November 9, 20 cc. of red blood cells were drawn from bull 136. As shown in experiment 5 this bull was infected with *B. bigemina* and *Anaplasma;* the infections being diagnosed 86 and 67 days previously to November 9. The cells were heated 10 minutes at 50° C. and 10 cc. were injected into each of the two bulls, 140 and 141. *Babesia bigemina* was not transmitted, but on December 16, 37 days after injection, both bulls came down with anaplasmosis; bull 140 died on December 23, 1935, and bull 141 died on January 2, 1936.

This experiment showed that exposure for 10 minutes at 54° C. killed the causative agents of piroplasmosis and anaplasmosis, whereas a like exposure at 50° C. killed the former agent but not the latter; it further showed that injection of the killed agents of the two diseases into 2 bulls failed to immunize them to anaplasmosis.

Experiment 7

On January 22, 1936, 10 cc. of red blood cells from steer 143, a mixed case of *Babesia bigemina* infection and anaplasmosis, diagnosed 30 and

9 days previously, were heated 15 minutes at 45° C. and injected into steer 142. On February 8, 17 days after this injection, the steer was positive for anaplasmosis and on February 25, 34 days after injection this animal was positive for *Babesia bigemina;* the latter infection was mild, without hemaglobinuria.

Exposure for 15 minutes at 45° C., therefore, failed to kill the causative agents of piroplasmosis and anaplasmosis but reduced the virulence of the former agent.

Experiment 8

On January 22, 10 cc. of red blood cells from steer 145 were heated 15 minutes at 45° C. and injected into steer 146 (steer 145 was infested 9 days previously with larvae of *Boophilus annulatus* and reacted to *Babesia argentina* infection diagnosed by splenic puncture on January 20; to *B. bigemina* on January 28; and to anaplasmosis on February 18). On January 30, 8 days after injection, steer 146 was positive for *Babesia argentina* (diagnosed by splenic puncture), and on February 27, 36 days after injection, this animal was positive for anaplasmosis; *B. bigemina* was apparently not transmitted.

In cells drawn during the incubation period of *Babesia bigemina* infection and anaplasmosis and during the clinical period of *B. argentina* infection, exposure for 15 minutes at 45° C. killed *Babesia bigemina* but did not kill *B. argentina* or *Anaplasma.*

Experiment 9

On February 19, 10 cc. of red blood cells from steer 143, a case of mixed infection with *Babesia bigemina* and *Anaplasma,* diagnosed 58 and 37 days previously, were heated 5 minutes at 46° C.; at the end of this period the cells were at 44.5° C. These heated cells were injected into steer 144, a case of anaplasmosis as noted in experiment 5. On February 25, 6 days after injection steer 144 was positive for *Babesia bigemina,* developed hemoglobinuria and died on February 27.

This experiment showed that exposure for 5 minutes at 46° C. failed either to kill *Babesia bigemina* or to reduce its virulence.

Experiment 10

On March 12, 10 cc. of cells from steer 145, a mixed infection of *Babesia argentina, B. bigemina,* and *Anaplasma,* diagnosed respectively 52, 44, and 23 days previously, were heated 10 minutes at 48° C. and injected into bull 148. On March 28, 16 days later, bull 148 developed anaplasmosis but not piroplasmosis, showing that exposure for 10 minutes at 48° C. killed *Babesia argentina* and *B. bigemina* but not *Anaplasma.*

The experiments on the *in vitro* heating of the etiological agents of all 3 of the above infections have been summarized in table 1.

TABLE I.—*Summary of experiments on heating the infected red blood cells in vitro to determine the subsequent behavior of the respective eitological agents of anaplasmosis and piroplasmosis in vivo*

Designation of bovine from which cells were drawn	Volume of packed cells	Temperature of bath	Period of heating	Period between diagnosis of infection and drawing of blood			Designation and age of bovine injected with infected cells		Incubation period of resulting infections			Degree of infection
				Babesia argentina	*Babesia bigemina*	Anaplasmosis			*Babesia argentina*	*Babesia bigemina*	Anaplasmosis	
No.	cc.	°C.	Minutes	Days	Days	Days	No.	Years	Days	Days	Days	
123	175*	60*	5–*	..	...	1 and 4*	127	2.5	..	..	33 or 36*	Mild
131	150	60	60	..	...	6	134	6+	..	..	..	.....
132	20	60	15	..	...	6	135	1.0	..	..	..	.....
130	25	60	15	..	30	214	137	6+	..	..	..	.....
136	10	57	10	..	32	13	135	1	..	..	..	.....
136	10	57	10	..	32	13	137	6+	..	..	..	.....
136	10	54	10	..	68	49	135	1	..	..	..	.....
136	10	54	10	..	68	49	137	6+	..	..	..	.....
130	10	54	10	..	82	266	140	6+	..	..	..	.....
130	10	54	10	..	82	266	141	6+	..	..	..	.....
136	10	50	10	..	86	67	140	6+	..	..	37	Fatal
136	10	50	10	..	86	67	141	6+	..	..	37	Fatal
136	10	48	10	..	106	87	135	1	..	..	..	.....
136	10	48	10	..	106	87	137	6+	..	..	..	.....
144	10	48	10	..	...	20	137	6+	..	..	33	Fatal
144	10	48	15	..	...	42	135	1****	..	..	29	Fatal
145	10	48	10	52	44	23	148	1	..	..	16	Severe
145	10	45	15	2	***	***	146	1.5	8	..	36	Mild
143	10	45	15	..	30	9	142	1.5	..	34	17	Mild
143	10	44.5**	5**	..	58	37	144	2	..	6	..	Fatal

* For explanation see experiment 2.
** For explanation see experiment 9.
*** Blood was drawn during the incubation period. See experiment 8.
**** Splenectomized before injection. See experiment 5.

DISCUSSION

One definite conclusion from the experiments herein reported is that the etiological agent of anaplasmosis, killed by heat, did not immunize susceptible cattle into which it was injected.

In five experiments in which the etiological agent of anaplasmosis survived either at 48° C., 50° C., or 60° C., the incubation period of the disease was lengthened from the normal of 15 days to 30 days or more; in other words, the normal incubation period was doubled but the virulence was not otherwise reduced. However, at 60° C. for a period less than 5 minutes the virulence of *Anaplasma* was greatly reduced. The experiments were complicated by the fact that three of the fatal cases were in old bulls and a fourth case occurred in a splenectomized bull; both age and splenectomy affected adversely the resistance of the bovine host to anaplasmosis.

Concerning the thermal death point of the causative agent of anaplasmosis, the following variable experimental factors occurred: (1) The temperature of the water bath, (2) the time of heating, (3) the quantity of cells heated, (4) the occurrence alone in the red blood cells of the agent of this disease or mixed with the agents of piroplasmosis and (5) the relative concentration of the former agent in the red blood cells. In the present writer's opinion the last of these variable factors was especially responsible for the contradictory results on the thermal death point, which appeared to be above 60° C. in one experiment and below 54° C. in all other experiments. Further experiments are needed to determine this point under conditions involving only one variable factor, the temperature of the bath for example. For this purpose it will be necessary to secure all of the samples of blood from clinical cases having a definitely determined percentage of red blood cells infected with *Anaplasma*. In demonstrating that the thermal death point of the etiological agent of anaplasmosis is higher than that of either of two agents of piroplasmosis the present writer has established a new method of securing pure infections of *Anaplasma* from mixed infections of *Piroplasma* and *Anaplasma*. Further experiments are needed to determine whether by this method infections with *Babesia argentina* may also be separated from those involving *B. bigemina*. For taxonomic studies and for immunization practices the value of such separation is obvious.

SUMMARY

1. The etiological agent of anaplasmosis was not killed by exposure for 18 hours at −78.6° C. and subsequent thawing at room temperature. The incubation period was lengthened but the virulence of the organism was not otherwise reduced.

2. None of five bulls was immunized as a result of one or more injections of red blood cells in which the etiological agent of anaplasmosis was killed by heat, whether this agent was present alone or mixed with the killed agents of piroplasmosis. All of the bulls contracted fatal infection of anaplasmosis when subsequently injected with the living agent.

3. In heavily infected red blood cells from a clinical case of anaplasmosis, some of the etiological agent of this disease survived *in vitro* for about 20 minutes during which the temperature of the cells was being raised from about 25° C. to 60° C. In all other tests this agent was killed at 60° C. and also at 57° C. and 54° C. In two tests it survived at 50° C.; in two tests it was killed at 48° C. but in three tests it survived at 48° C.

4. In one case of survival at 60° C., the etiological agent of anaplasmosis showed loss of virulence; in all cases of survival at temperatures lower than 60° C., the incubation period was lengthened but the virulence was not otherwise reduced.

5. In one case, after an incubation period of 36 days, anaplasmosis was transmitted by cells drawn from the "donor" during the incubation period and heated at 45° C. for 15 minutes.

6. *Babesia argentina* and *B. bigemina* were killed in cells heated 10 minutes in a bath at 48° C. and *B. bigemina* at all temperatures higher than 48° C.; they both survived at 45° C.

7. The differential between the thermal death points of the etiological agents of anaplasmosis and piroplasmosis has furnished the basis of a practicable method of separating the two infections.

8. The virulence of *Babesia bigemina* was greatly reduced by heating for 15 minutes at 45° C.

STUDIES ON *DIENTAMOEBA FRAGILIS* (PROTOZOA)

II. REPORT OF UNUSUAL MORPHOLOGY IN ONE CASE WITH SUGGESTIONS AS TO PATHOGENICITY

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I. INTRODUCTION

In a recent paper (Wenrich, 1936) attention was called to the fact that the supposedly rare intestinal amoeba of man, *Dientamoeba fragilis*, has a higher incidence of infection, a greater viability outside the host and a greater range in size of body and in structure of the nucleus than had previously been reported.

In regard to structure it was noted that the original description given by Jepps and Dobell (1918) has largely been confirmed by subsequent observers but that there have been differing interpretations of the organization of the nucleus.

Concerning its structure it was pointed out that the vesicular nucleus has a delicate membrane, usually without noticeable chromatin granules upon it; within this membrane is a clear zone surrounding a central mass which Jepps and Dobell called the "karyosome" and which Kudo (1926) referred to as the "nucleolus." This central mass is thought to consist of a matrix in which are imbedded a series of chromatic granules. The number of these granules is commonly four, as pointed out by Craig (1926), but may be more. Jepps and Dobell noted that frequently one of the granules is somewhat larger and more intensely stained than the others and I have interpreted this more heavily-staining granule as the true karyosome, or, better, endosome, and have interpreted the other granules as equivalent to the periendosomal granules of other amoebae.

Attention was also called to the occurrence of larger mononucleate amoebae with enlarged nuclei, more chromatic membranes, usually with more than four granules and often with large and prominent endosomes. However, in some of these enlarged amoebae, the endosomes remain relatively small.

It is the purpose of the present communication to describe the unusual morphology, especially in regard to nuclear structure, in the amoebae of one particular case and to add to the suggestions of Gittings and Waltz (1927) and of Hakansson (1936) that *Dientamoeba fragilis* may have pathogenic properties.

The preparation of this paper has been aided by a grant from the Special Research Fund of the University of Pennsylvania.

I am very much indebted to Dr. A. D. Waltz, Pathologist at the

Children's Hospital, Philadelphia, Pa., for his hearty cooperation in connection with this and many other cases. Dr. Waltz very kindly prepared the history of the case given below. This is case 1 of the paper by Gittings and Waltz (1927). During the early part of this study the girl was a case in the private practice of Dr. Gittings, Dr. Waltz handling the laboratory procedures. After the family moved away Dr. Waltz was called upon from time to time to make blood counts and stool examinations.

II. HISTORY OF THE PRESENT CASE

Poorly nourished during the first year of life, tonsils and adenoids were removed at age two because of recurring attacks of otitis media. Pyelitis occurred at three, varicella at five, measles at six and mumps at seven; not subject to diarrhea but to constipation.

At age five (Nov. 1925), came under the observation of Dr. Gittings because of lack of energy, aggravated anorexia and slight evening temperature, symptoms which had persisted for several weeks. Physical examination essentially negative except for evidences of malnutrition and fatigue posture; tuberculin test negative; blood count: hemoglobin, 78%; erythrocytes, 5,390,000; leucocytes, 16,200; neutrophiles, 32%; lymphocytes, 48%; monocytes, 2% and eosinophiles, 18%; second blood count essentially the same with eosinophiles at 16%; stool examination showed *Dientamoeba fragilis* in great numbers. After two courses of treatment with emetin-bismuth-iodide, amoebae decreased in number and oesinophiles dropped to 7%. During next two years a few *Dientamoeba* persisted; eosinophiles ranged from 7% to 11%, and patient remained undernourished. At this time (1927) patient moved from the city; not seen until four years later when she reported for further examination. The child's mother, who is a trained nurse, gives an account of conditions during these four years.

During the first three years the child had been gaining slowly; then one morning she awakened suddenly, vomited and the bowels moved. This was followed almost immediately by a severe chill which lasted for ten minutes; the temperature rose rapidly to 108 degrees at the end of five hours, then dropped fairly rapidly to 104 degrees, where it held for two days; during this period there were no bowel movements and no peristalsis could be heard. Next day bloody, watery discharge from the bowel contained a mucous cast about fourteen

Table showing results of fourteen blood counts

Date	Erythrocyte count	Leukocytes, total	Hemoglobin %	Neutrophiles %	Lymphocytes %	Monocytes %	Eosinophiles %	Basophiles %
11/16/25	5,390,000	16,200	78	32	48	2	18	0
11/24/25	5,250,000	18,600	83	54	26	4	16	0
12/14/25	5,490,000	19,800	85	40	53	0	7	0
1/ 4/26	5,100,000	5,800	86	48	38	3	11	0
2/ 2/26	5,140,000	8,900	78	53	34	4	9	0
3/ 5/26	4,770,000	8,200	82	58	25	6	11	0
3/23/26	5,110,000	7,500	84	46	41	3	10	0
5/21/26	5,380,000	7,400	80	51	35	4	10	0
7/ 1/26	———	19,000	—	57	28	4	10	1
5/ 6/27	4,900,000	8,900	86	54	35	4	7	0
4/ 4/31	5,360,000	5,700	85	71	25	1	3	–
11/19/31	4,700,000	9,000	90	49	47	2	1	1
6/29/33	5,260,000	6,100	90	40	51	6	3	0
6/28/28	4,630,000	7,400	94	59	33	3	2	1

inches long. The blood amounted to a frank hemorrhage during this day when there were numerous bloody, watery stools. The diarrhea gradually decreased to normality at the end of five days. The family physician gave a diagnosis of toxic colitis following food poisoning. This was difficult to substantiate since several

other children eating the same food remained symptomless. The stools were not cultured for bacteria nor examined for parasites. Three months later she complained of pain in the right upper quadrant of the abdomen. This was largely relieved by a cathartic, but felt subnormal for the next week. Pain and tenderness began to increase with slight swelling below the right costal margin; temperature rose to 102 degrees. A duodenal drainage gave no relief. An X-ray examination at a local hospital left the diagnosis doubtful; treated expectantly for two weeks on a liquid diet; there was no diarrhea and the temperature ranged from 101 to 104 degrees; became markedly wasted and dehydrated. An operation was performed and on opening the abdomen, 200 to 300 cc. of thin creamy pus escaped. Drainage continued for two days with a tube and for two weeks without the tube. No culture for bacteria nor examination for amoebae was made. Diagnosis was traumatic abscess of the liver; there had been a fall with slight injury to the area over the ribs but without any external evidence of injury. Temperature fell to normal after operation. During the latter part of 1931 the patient was treated for amoebiasis by the hot-water injection method.

After this period a stool examination revealed a few *Dientamoeba.* A course of treatment with yatren was continued for three weeks without interruption. Since that time several negative stool examinations have been made. Eosinophiles are now 2%. The accompanying table shows the results of fourteen different blood counts.

Lacking an opportunity to study the stools during the acute febrile attack with diarrhea or the pus from the abscess, one can only conjecture as to the part played by *Dientamoeba,* although suggestive.

During routine study of stools, five other patients have been found to have eosinophiles ranging from 6% to 21% asociated with *Dientamoeba.* In two of these no other possible cause of eosinophilia was found. One case carried *Giardia,* one *Endolimax nana* and one a suggestive allergic history. A larger number of carriers of *Dientamoeba* had no eosinophilia.

III. VARIATIONS IN THE MORPHOLOGY OF THE AMOEBAE

A. General features. In the previous paper (Wenrich, 1936) some unusual morphological conditions in *Dientamoeba* were noted, especially the large mononucleate individuals with enlarged nuclei and usually with enlarged and prominent endosomes. In the present case there were many more of these unusual amoebae and consequently more variations of the type. Also large and unusual nuclei occurred in small and medium-sized animals as well as in larger ones. There was a high percentage of mononucleate individuals and, in addition, definitely degenerate amoebae and conditions interpreted as abortive stages in mitosis and stages in unequal nuclear division were recognized. The sizes ranged up to 20 μ in diameter.

Eight times during the study of this case by Drs. Gittings and Waltz, and later by Dr. Waltz, I was supplied with fecal specimens for study. The morphology of the amoebae varied somewhat with the different fecal samples obtained. The following set of notes will indicate some of these differences.

1. Nov. 24, 1925. Feces 8 or 9 hrs. old; fixed in Schaudinn's fluid plus a little acetic acid; nuclear staining so faint that no counts of mononucleates and binucleates could be made; many degenerate and unidentifiable bodes on the slides.

2. Nov. 27, 1925. Feces 2 or 3 hrs. old; fixed in Schaudinn's plus a little acetic, modified Bouin's (picric, 75, formol, 15, acetic, 10 parts) and Hollande's fluids; nuclei not stained with first but satisfactorily stained with the other two fixing solutions; most of the amoebae normal in appearance, but there were a few of the large type; mononucleates, 27 per cent.

3. Nov. 30, 1925. Feces several hrs. old; fixed in Schaudinn's plus a little acetic and in Hollande's fluids; most of the amoebae had unusual nuclei, 63 per cent being mononucleate. (All drawings were made from these slides.)

4. Dec. 10, 1925. No amoebae found.

5. Jan. 4, 1926. Smears fixed in Schaudinn's plus a little acetic and in modified Bouin's fluids. Most of the amoebae had unusual nuclei, 91 per cent being mononucleate.

6. Jan. 9, 1926. No amoebae found.

7. Mar. 23, 1926. Feces 4 hrs. old; fixed in Schaudinn's fluid plus a little acetic and in modified Bouin's; many amoebae normal, also many with unusual nuclei, 54 per cent being mononucleate.

8. Apr. 4, 1931. Feces several hrs. old; fixed in Schaudinn's plus 5 per cent of acetic and in same plus 15 per cent of acetic, besides modified Bouin's; most amoebae were normal although some had enlarged nuclei; 43 per cent were mononucleate. At this time it was learned that the addition of larger amounts of acetic acid to Schaudinn's fluid would result in fixation giving satisfactory staining of nuclei. This fact has recently been noted by Johnson (1935).

From the above notes it will be seen that in some stools most of the amoebae were normal while in others most were unusual as to nuclear structure. Whether these differences could be correlated with drug action in connection with the treatments received by the host could not be determined because the treatment records were not available at the time this paper was written.

B. Variations in nuclear structure. The unusual nuclear conditions found in this case and illustrated in figures 1–32 can best be explained on the assumption that variations in nuclear appearance may be due either to preparation for nuclear division or to physiological conditions other than those associated with division. These physiological conditions are, of course, the resultants of the effects of the environment upon the organization and functioning of the protoplasm.

It may also be postulated that each of the morphological features of the nucleus may vary to a different degree and in a different way from the others. Thus: the nucleus as a whole may be larger or smaller in relation to the size of the cell; its membrane thicker or thinner; the central mass larger or smaller with respect to the whole nucleus; the endo-

some larger or smaller with respect to nuclear size and with respect to the periendosomal granules; and the latter may vary so that they may be larger or smaller, or more or less numerous, or more heavily or more lightly stainable, or they may tend to lose their identities. All of these possibilities seem to be realized in the present case as shown in the accompanying illustrations, which also depict some changes incident to nuclear division.

Variation in nuclear size is represented by a range in diameter from less than 2 μ to more than 4 μ. Although there is a general tendency for nuclear size to be correlated with cell size there are noteworthy exceptions. For example, figure 12 illustrates an amoeba 8 μ by 7 μ with a nucleus about 4 μ in diameter while figure 10 represents a large amoeba, 21 μ by 16 μ with a nucleus only sligthly more than 3 μ in diameter.

There is a tendency for the nuclear membrane to increase in thickness with increase in size, although there is some variation in this respect. In some cases the membrane appears to be incrusted with a thin layer of chromatic material (Fig. 15).

The central nuclear mass which includes the endosome and its accompanying periendosomal granules, is subject to wide variation in relative size in comparison to the nucleus as a whole. In most cases the central mass has a diameter about 0.4 that of the nucleus but in figure 6 the ratio is 0.67 and in figure 7 it is 0.7.

As noted in the previous study, and illustrated here by figures 3, 7–17 and 22–24, there is a tendency for the endosome to become enlarged and to become more distinctly differentiated from the other granules. In some cases, however, the endosome remains relatively small (Figs. 3, 4–6, 20 and 21). The shape is also subject to divergence from the more common rounded form (Figs. 3, 6–8 and 17).

There seem to be several types of variation for the periendosomal granules. Without change in number, they may become much enlarged along with the rest of the nucleus (Fig. 12), or the number may become increased (Figs. 6, 7, 9–11, 13, 15, 21–23). In many cases they appear to lose their identity in a more or less homogeneous mass (Figs. 2, 5–8, 18–20).

To what extent these variations in nuclear morphology are to be interpreted as degenerate is difficult to decide, but figures 16 and 17 indicate conditions that could not well be interpreted otherwise. In figure 16 the nuclear membrane has broken down on one side and in figure 17 the endosome has become flattened against the nuclear membrane, which has thinned out on the opposite side.

C. Cytoplasmic degeneration. In addition to nuclear degeneration there are also evidences of cytoplasmic degeneration. For example, the more homogeneous areas at the right in figures 24 and 27 appear to be

degenerate. Pseudopodial areas have a different appearance, as seen in figures 14, 16, 18 and 21.

D. Nuclear division. When a large number of animals are available for study one naturally looks for instances of division. Considering the preponderance of apparently abnormal conditions in the material from fecal sample no. 3 from the present case and illustrated in the figures, one might expect to see abnormal nuclear divisions. Figures 25 and 26 are interpreted as representing individuals in each of which a hypertrophied nucleus has attempted to form chromosomes for divisions. Figures 28 and 29 probably represent more nearly normal anaphases. Figure 27 may or may not represent a prophase leading toward such a division. Figures 30–32 show late stages of unequal, and therefore abnormal, nuclear divisions.

Altogether the range of variation in morphology of the nucleus is unexpectedly great.

IV. DISCUSSION

A. *Dientamoeba fragilis* the only amoeba present. When the study of the slides prepared from this case was first begun the question arose as to whether or not there might be some *Iodamoeba* along with the *Dientamoeba* (see illustrations). Because of lingering doubt as to the identity of some of the more bizarre forms, publication of the results has long been delayed. The decision that only *Dientamoeba* was present was influenced by the following considerations:

1. Although more than a dozen examinations were made by Dr. Waltz and myself, no typical specimens of any other amoeba were ever discovered.

2. When the series of variants were studied together, gradual transitions could be found from the typical condition (Fig. 1) to the more diverse variants.

3. In spite of wide variations in nuclear conditions, the cytoplasmic structure remained essentially the same and was typical of *Dientamoeba*.

4. The typical staining reactions applied to all forms regardless of nuclear structure: after Schaudinn's fluid with only a little acetic acid added the nuclei stained very poorly or not at all, while staining well after modified Bouin's, Hollande's or Schaudinn's with 15 per cent of acetic added. This would not be the case for any other species.

5. In the subsequent study of prepared slides from about 70 cases of *Dientamoeba* infection, many instances were found where variants showed fundamental resemblances to the more extremely divergent conditions revealed in the present case.

All the evidence, therefore, indicates that *Dientamoeba* was the only amoeba present in this case.

B. Possible origin of unusual conditions. As to the causes which have produced the unusual conditions revealed in this study, one can only speculate. Since the same type of variation is not uncommon, although not usually so extreme as in the present case, some of the causes must be equally common. Protozoologists generally recognize that much degeneration is to be expected in the trophozoite stages of intestinal protozoa passed in the feces and *Dientamoeba* is supposed to be especially subject to rapid degeneration outside the host. It has been shown that some individuals of this species have considerable viability outside the host (Wenrich, 1936; Hakansson, 1936) hence it would be interesting to determine which variations are the more viable.

Mononuclearity seems to be correlated with the unusual nuclear structures here reported. I have previously suggested (1936) that large mononucleate individuals may arise from loss of capacity for nuclear division while retaining the ability to feed and grow; also that nuclear fusion may sometimes result in mononuclearity. In the present study there is evidence that one nucleus may degenerate. In figures 5 and 6, for example, there are areas below the definitive nuclei which may be degenerating nuclei. If in some cases, as shown in figures 30–32, unequal division may take place, then the smaller nucleus might degenerate for lack of a full complement of chromatin elements. The remaining nucleus might then persist as a hypertrophied and abnormal component of the cell.

A high percentage of mononucleate amoeba may be correlated with special conditions in the digestive tract. Kudo (1926) reported 87.8 per cent mononucleates in a case of dysentery where *Endamoeba histolytica* was not discovered.

C. Possible pathogenicity of *Dientamoeba fragilis*. Hakansson (1936) has recently raised the question as to the pathogenicity of this species, evidently overlooking the prior suggestion of Gittings and Waltz (1927). While the possible existence of *Endamoeba histolytica* can not be absolutely ruled out in the present case, if it had been present, it should have been found in the more than a dozen examinations that have been made. Dr. Waltz notes that other cases have shown high percentages of eosinophiles associated with the presence of *Dientamoeba*. A number of other cases of *Dientamoeba* infection with more or less noticeable symptoms have occurred in my own experience and in our survey of college students (Wenrich, Stabler and Arnett, 1935) it was found that the hosts infected with *Dientamoeba fragilis* recorded somewhat more gastro-intestinal disturbances than those harboring *Endamoeba histolytica*.

Over a period of years I have had occasion to make a good many stool examinations for one particular host. During these years *Dienta-*

moeba was found during three different periods. Previous to July, 1921, this species had not appeared in the stools of this host although *Endolimax* and *Chilomastix* had. These three organisms continued to appear in the stools through the remainder of 1921 but did not appear in examinations made in 1922 and in 1923. In 1924 *Dientamoeba* appeared again during an attack of diarrhea but the other two protozoa did not. This amoeba continued to appear through 1924, 1925 and into the spring of 1926 and then disappeared. No other protozoon was found during this second period of infection. This amoeba did not appear again until in December, 1928, when a period of mild diarrhea again stimulated more frequent stool examinations. No other protozoon was found but *Dientamoeba* continued to appear up to the spring of 1931. Since then it has not reappeared in the stools of this host.

Each of these three periods of infection with *Dientamoeba* began with mild but definite diarrhea and during the infective periods, especially during the early part of each, there was marked susceptibility to colds and attacks of influenza. It is recognized, of course, that such conditions as diarrhea and tendency to colds may be caused by many other agents, but the three periods just described are so consistent in the association of *Dientamoeba* with mild disturbances of the large intestine along with susceptibility to disturbances of the respiratory passages, that it is believed that the correlation is significant.

It is suggested that protozoologists and clinicians may well look for other cases of low vitality, high eosinophilia and more or less definite disturbances of the large intestine associated with the presence of *Dientamoeba.* In this connection it should be borne in mind that *Dientamoeba* does not form cysts and is therefore not usually found in formed stools but only in soft feces. It should further be remembered that it is a rather difficult species to recognize in the fresh condition, but is more readily detected on properly prepared slides. If Schaudinn's fluid is used as a fixative it should have at least 10 per cent of glacial acetic acid added to insure stainability of the nuclei.

V. SUMMARY

1. A history, provided by Dr. A. D. Waltz, is given of a girl who had poor health in early life; at age of five examination by Dr. J. C. Gittings showed lack of energy, aggravated anorexia and slight temperature. A blood examination showed 18 per cent eosinophiles. Stool examination by Dr. Waltz showed large numbers of *Dientamoeba fragilis.* Treatment reduced the numbers of amoebae and the percentage of eosinophiles. The family then moved away but vitality of the patient continued to be subnormal. At age of ten there was an attack of colitis followed about four months later by an operation for liver abscess. At that time

there was no examination for parasites. Later a treatment by the hot water injection method did not remove *Dientamoeba.* They disappeared after treatment with yatren. Eosinophiles then became normal.

2. Through the kindness of Dr. Waltz, I was able to make eight stool examinations in this case. Dr. Waltz made many other examinations and *Dientamoeba* was the only parasite ever found by either of us.

3. In some of the stools examined, study of fixed and stained slides showed that the amoebae were largely typical while in other stools they were largely atypical, including sizes up to 20 μ in diameter and a great variation in nuclear structure. Mononucleates ranged from 27 per cent to 91 per cent.

4. Each of the morphological features of the nucleus seemed to be capable of varying to a different degree and in a different way as compared to the others. The nucleus as a whole could be large or small in relation to the size of the cell; its membrane could be thicker or thinner; the central mass could be larger or smaller with respect to the whole nucleus, the endosome larger or smaller with respect to nuclear size and with respect to the periendosomal granules, and the latter could vary so that they were larger or smaller, more or less numerous, more or less stainable or more or less distinctly identifiable.

5. It is difficult to determine how much of this variation is to be considered abnormal. However, some amoebae that were definitely degenerate both as to nucleus and cytoplasm could be recognized.

6. Abortive nuclear division and unequal nuclear divisions were observed. It is suggested that the latter process could be followed by degeneration of the smaller nucleus and abnormal hypertrophy of the larger one.

7. The history of the present case suggests a pathogenic role for *Dientamoeba fragilis.* Dr. Waltz reports other cases of correlation between eosinophilia and the presence of *Dientamoeba.* In my own experience a good many cases of low vitality have been associated with the presence of *Dientamoeba.* In one case, over a period of ten years, one individual had three periods of coincident presence of *Dientamoeba* with initial diarrhea and a tendency to colds and influenza. It is therefore suggested that other workers watch for evidence of a possible pathogenic role for *Dientamoeba fragilis.*

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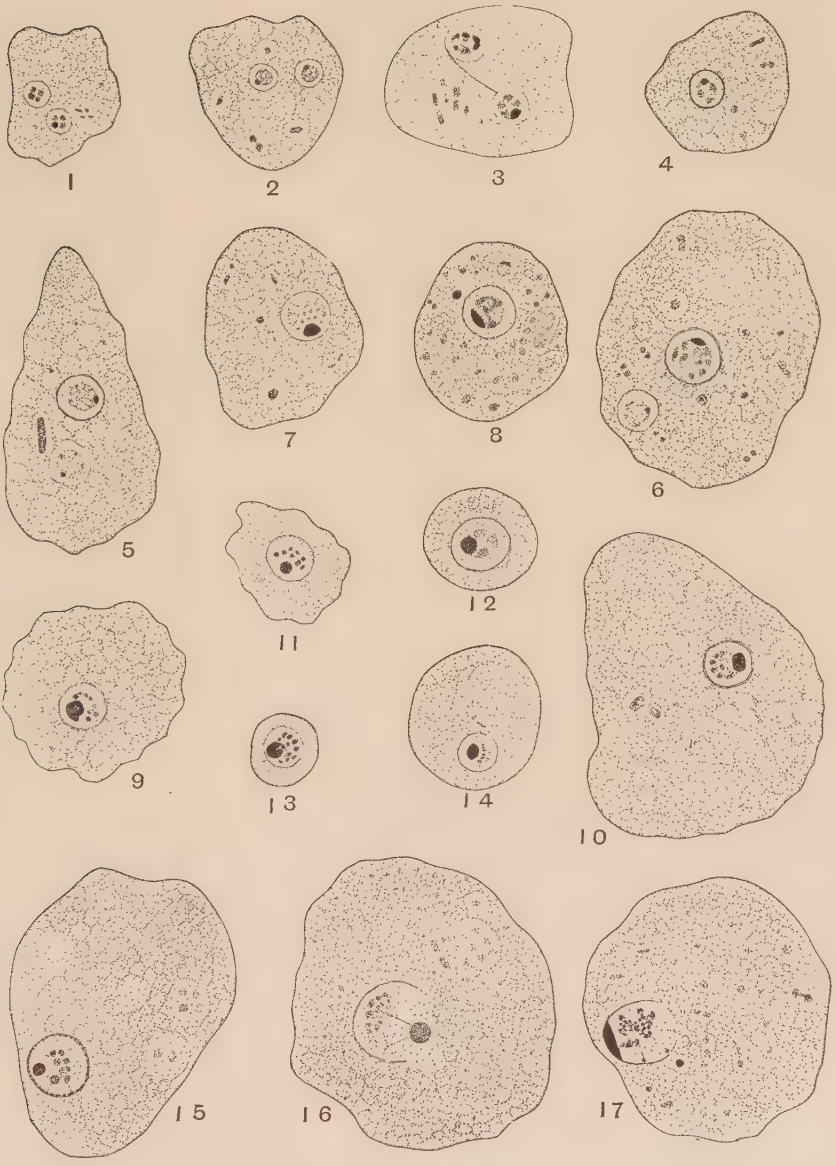
PLATE I



VII. Explanation of Figures

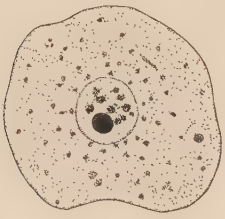
All drawings have been made by the aid of a camera lucida from smears fixed in Hollande's fluid and stained with iron alum hematoxylin. Drawn originally at a magnification of ×3000 they have been reduced about one-third in printing.

Plate I

Fig. 1. Individual of normal size with normal nuclei.
Fig. 2. Slightly larger individual with nuclei showing distinct endosomes but with periendosomal granules indistinct.
Fig. 3. Still larger individual with desmose connecting the two nuclei; endosomes distinct, other granules less so.
Fig. 4. Smaller mononucleate individual with nucleus similar to those in figure 3.
Fig. 5. Large individual with single nucleus like those in figure 2; endosome small, other granules unidentifiable; possible degenerating nucleus below the single one.
Fig. 6. Large individual with enlarged nucleus and possibly degenerating nucleus below it; large central area, small endosome, other granules less distinct.
Fig. 7. Medium large individual; nucleus with unusually large central area; endosome large, other granules numerous and partly indistinct.
Fig. 8. Medium large individual; large nucleus with large endosome, three other granules partly coalesced.
Fig. 9. Medium large individual; nucleus with large endosome and relatively small periendosomal granules.
Fig. 10. Large individual, 21 μ by 16 μ, with relatively small nucleus; endosome relatively large, other granules less distinct.
Fig. 11. Small individual with large nucleus; all granules distinct.
Fig. 12. Small individual with very large nucleus, large endosome, the three other granules enlarged but still identifiable.
Fig. 13. Very small individual with homogeneous cytoplasm, relatively large nucleus, large endosome and numerous other granules.
Fig. 14. Normal-sized individual, large endosome, other granules not distinct.
Fig. 15. Large individual; large nucleus, endosome detached from group of smaller granules; thin layer of chromatic material against the membrane. (*Cf.* Fig. 11.)
Fig. 16. Very large individual with nucleus degenerating; nuclear membrane has broken down on the right.
Fig. 17. Large individual with degenerating nucleus; endosome a peripheral plaque, other granules breaking up, membrane thinning on the right.

PLATE II

18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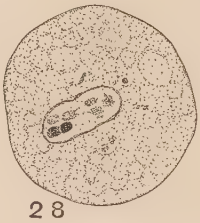
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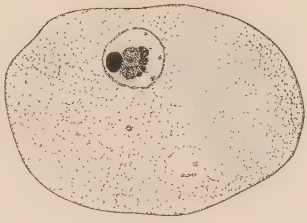
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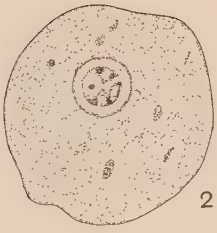
29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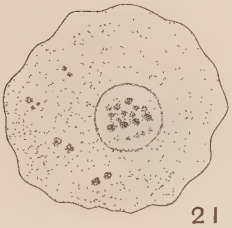
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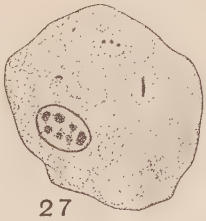
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Plate II

Fig. 18. Fairly large binucleate individual; granules indistinct, endosomes small.
Fig. 19. Mononucleate individual with small nucleus, granules indistinct.
Fig. 20. Individual with larger nucleus, more chromatic membrane, small endosome, other granules indistinct, central area distinctly delimited.
Fig. 21. Individual with large nucleus, central area not clearly delimited, endosome small.
Fig. 22. Mononucleate individual with view of nucleus which suggests a central endosome surrounded by a layer of periendosomal granules.
Fig. 23. Mononucleate individual with large nucleus, large endosome and numerous other granules.
Fig. 24. Probably degenerating individual; degenerate cytoplasm at the right, large endosome and clumped periendosomal granules.
Figs. 25 and 26. Individuals with large nuclei containing deeply-staining masses simulating chromosomes.
Fig. 27. Individual with degenerate cytoplasm on the right, elongated nucleus, possibly in prophase of division.
Figs. 28 and 29. Individuals with elongated nuclei, probably in anaphase of division.
Figs. 30–32. Individuals showing telophases of unequal and therefore abnormal nuclear divisions.

OCCURRENCE OF HAEMATOZOA IN SOME CALIFORNIA BIRDS AND MAMMALS

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INTRODUCTION

The blood of 203 birds, comprising 21 species, and of 185 mammals, including 18 species and subspecies, has been examined for haematozoa by the writers. These studies were started in 1930 at the University of California, Berkeley, and are being continued at the University of California at Los Angeles. The writers are indebted to Mr. and Mrs. Harold Michener, of Pasadena, for many of the birds examined, and to Mr. Kenneth Stager, of Los Angeles, for all but one of the bats. The haematozoa observed have not all been identified as to species because the necessary developmental stages were not available. In most cases both stained and fresh blood samples were examined. In the case of a few mammals only fresh preparations were made.

BIRDS

One of the most interesting infections encountered was that of a Russet-backed Thrush (*Hylocichla u. ustulata*) which was found in a dying condition on the steps of the Life Sciences Building on the Berkeley Campus. This bird had a quadruple infection with *Haemoproteus* (figs. 7, 11), *Trypanosoma, Leucocytozoon* and a microfilarial worm. Coatney (1936) in his check-list and host-index of the genus *Haemoproteus* records this parasite from eight species of thrushes from various countries. From these eight hosts, two species of *Haemoproteus* have been designated, *H. geocichlae* from *"Geocichla lunulata,"* and *H. danilewskii* from *"Turdus merula."* Comparative studies should be made to clarify the specific status of these parasites.

The trypanosome (fig. 9) from the thrush is a broad organism with parallel striations running lengthwise of the body. It resembles *Trypanosoma gallinarum* from the Uganda Fowl as figured by Wenyon (1926). It is also similar to the trypanosomes of birds from the South Seas described by Uegaki (1930, figs. 64–70) except that in the present case the nucleus does not take the stain. Wenyon points out that trypanosomes of birds may be very polymorphic and that it is unwise to name a species until something is known of its life cycle. All of the organisms in the thrush were, however, much like figure 9 in size and general morphology.

There were two stages of *Leucocytozoon* noted in the thrush. They

EXPLANATION OF FIGURES

All figures drawn with the aid of the camera lucida. Magnification, × 1235.

Fig. 1. *Trypanosoma neotomae* and erythrocyte from the Wood Rat, *Neotoma fuscipes annectens.*

Fig. 2. *Trypanosoma* sp. from the Audubon Warbler, *Dendroica a. auduboni.* With May-Giemsa, the cytoplasm stains pale blue. The undulating membrane is colorless, the axoneme pale blue. At the base of the undulating membrane is a row of violet granules. The parabasal stains purple. The nucleus consists of a group of faintly-staining violet granules.

Fig. 3. *Trypanosoma cruzi* in experimentally infected House Mouse, *Mus musculus.*

Fig. 4. *Trypanosoma neotomae* and erythrocyte from the Wood Rat, *Neotoma fuscipes macrotis.*

Fig. 5. *Trypanosoma microti* and erythrocyte from Meadow Mouse, *Microtus californicus sanctidiegi.*

Figs. 6, 10. *Haemoproteus* sp. from House Finch, *Carpodacus mexicanus frontalis.* Fig. 6, male gametocyte, stains pale blue with Jenner-Giemsa, has loosely assembled, pink-staining nucleus. Fig. 10, female gametocyte, stains deep blue and has well-defined, pink-staining nucleus. Both male and female cells show refractile, blackish pigment granules.

Figs. 7, 11. *Haemoproteus* sp. from Russet-backed Thrush, *Hylocichla u. ustulata.* With Jenner-Giemsa the cytoplasm appears light blue and granular. The nuclei stain light pink. There are refractile, brownish-black pigment granules. Note two parasites in fig. 7.

Fig. 8. Microfilaria and normal erythrocyte from *H. u. ustulata.*

Fig. 9. *Trypanosoma* sp. from *H. u. ustulata.*

Fig. 12. Intraleucocytic *Toxoplasma* sp. from House Finch, *Carpodacus mexicanus frontalis.* With May-Giemsa the slightly granular cytoplasm stains light blue. The central clump of granules stains pink. The nucleus of the host cell appears dense and pycnotic.

are identical in morphology with those described by Uegaki (1930, figs. 35–40). In both cases the nucleus of the host cell is distorted, forming a narrow band partially surrounding the parasite. With Jenner-Giemsa stain the oval macrogametocyte stains deep blue, has a nucleus composed of pale violet granules and an adjacent deep violet oval granule with a vacuole at one end. The size range is approximately 13×10 microns. The microgametocyte stains light blue and has a large oval nucleus composed of pale pink granules. The adjacent, oval violet body of the macrogametocyte is not present in the microgametocyte. The size is approximately 7×10 microns.

The microfilaria (fig. 8) is an unsheathed form and was fairly numerous in the blood of the thrush. Its affinities are not known.

A light infection with a trypanosome (fig. 2) was noted in an Audubon Warbler (*Dendroica a. auduboni*) from Pasadena. Only three trypanosomes were seen in two stained blood smears, each covering about half of an ordinary 3 by 1 inch microscope slide.

Five House Finches (*Carpodacus mexicanus frontalis*), two from Pasadena and three from Los Angeles, harbored a species of *Haemoproteus* (figs. 6, 10). The forms seen were gametocytes of typical halteridium shape, and were fairly numerous in the blood of three of the birds. According to Coatney (1936), no *Haemoproteus* has been described from House Finches.

Two other House Finches from Los Angeles showed *Toxoplasma,* an intraleucocytic organism (fig. 12). It is an oval body with a group of pinkish-staining granules in the center. The nucleus of the leucocyte shows the effect of pressure from this body. Only three such organisms were seen in two smears, one from each bird.

The following birds from Los Angeles County were negative for Haematozoa: 6 Brewer Blackbirds (*Euphagus cyanocephalus*) from (3) Los Angeles and (3) Pasadena; 1 Coast Bush Tit (*Psaltriparus m. minimus*), 1 Bailey Mountain Chickadee (*Penthestes gambeli baileyae*), 12 Dwarf Cowbirds (*Molothrus ater obscurus*), 5 Chinese Spotted Doves (*Spilopelia c. chinensis*), 3 California Jays (*Aphelocoma c. californica*) and 1 Slate-colored Junco (*Junco h. hyemalis*) from Pasadena; 120 House Finches (*Carpodacus mexicanus frontalis*) from (85) Pasadena and (35) Los Angeles; 1 California Purple Finch (*Carpodacus purpureus californicus*), 1 Willow Goldfinch (*Spinus tristis salicamans*) and 1 Anna Hummingbird (*Calypte anna*) from Pasadena; 7 Western Mockingbirds (*Mimus polyglottis leucopterus*) from (4) Los Angeles and (3) Pasadena; 1 Lincoln Sparrow (*Melospiza l. lincolnii*) and 3 Gambel Sparrows (*Zonotrichia leucophrys gambelii*) from Pasadena; 17 English Sparrows (*Passer domesticus*) from (16) Pasadena and (1) Los Angeles; 4 San Diego Song Sparrows (*Melospiza melodia cooperi*) from

(2) Los Angeles and (2) Pasadena; 2 Anthony Brown Towhees (*Pipilo crissalis penicula*), 2 San Diego Spotted Towhees (*Pipilo maculatus megalonyx*), 2 California Thrashers (*Toxostoma r. redivivum*), 1 Russet-backed Thrush (*Hylocichla u. ustulata*) and 3 Audubon Warblers (*Dendroica a. auduboni*) from Pasadena.

MAMMALS

Trypanosoma neotomae (figs. 1, 4) was discovered in twelve Portola Wood Rats, *Neotoma fuscipes annectens,* from Berkeley, Alameda County, one San Diego Wood Rat, *N. f. macrotis,* from Los Angeles County and one Desert Wood Rat, *N. l. lepida,* from near Victorville, San Bernardino County. Cross inoculations showed the trypanosomes of the first two mentioned subspecies of rats to be the same (Wood, 1936). The trypanosome from *N. l. lepida* appears to be identical. *Trypanosoma neotomae* is of the *lewisi* type, but attempts to infect Norway Rats, the natural hosts of *T. lewisi,* with blood of infected wood rats were unsuccessful, indicating a physiological difference between the two trypanosomes.

One Wood Rat from San Diego County was found infected with *Trypanosoma cruzi* (fig. 3), the cause of Chagas' disease or American human trypanosomiasis.

A Meadow Mouse, *Microtus californicus sanctidiegi,* from San Diego County, carried an infection with lewisi-like *Trypanosoma microti* (fig. 5). Two young albino rats (*Rattus norvegicus*) and a White-footed Mouse (*Peromyscus californicus insignis*) inoculated with blood of the Meadow Mouse failed to become infected. The *Microtus* showed a fairly heavy infection for 11 days, after which the trypanosomes gradually disappeared from the blood. No dividing trypanosomes were seen. On the 22nd day after capture the mouse died. It is believed that this infection represents a new host record for *T. microti* and also the first record of its presence on the Pacific coast. Originally described from *Microtus arvalis* in France, Coatney (1935) has recently reported it in *Microtus pennsylvanicus.*

The mammals examined which were negative for Haematozoa are as follows: 50 Portola Wood Rats (*Neotoma fuscipes annectens*) from (49) Berkeley, Alameda County, and (1) Ben Lomond, Santa Cruz County; 75 San Diego Wood Rats (*N. f. macrotis*) from San Diego County; 1 Norway Rat (*Rattus norvegicus*) from Alameda County; 3 Parasitic Mice (*Peromyscus c. californicus*) from Berkeley, Alameda County; 14 Southern Parasitic Mice (*Peromyscus californicus insignis*) from (8) San Diego County and (6) Los Angeles County; 2 San Diego Desert Mice (*Peromyscus eremicus fraterculus*) from (1) Los Angeles County and (1) San Diego County; 5 Gambel White-footed Mice (*Pero-*

myscus maniculatus gambelii) from (3) Los Angeles County and (2) Alameda County; 5 Gilbert White-footed Mice (*Peromyscus truei gilberti*) from Berkeley, Alameda County; 1 West-central California Meadow Mouse (*Microtus c. californicus*) from Berkeley, Alameda County; 1 Pocket Gopher (*Thomomys* sp.), Calaveras County; 1 Desert Antelope Ground Squirrel (*Ammospermophilus l. leucurus*), 1 Allen Pocket Mouse (*Perognathus californicus dispar*), and 1 Kangaroo Rat (*Dipodomys* sp.) from Los Angeles County; 1 Mexican Free-tailed Bat (*Tadarida mexicana*) from Alameda County; 1 Pacific Pallid Bat (*Antrozous pallidus pacificus*) from Azusa, Los Angeles County; 8 California Mastiff Bats (*Eumops perotis californicus*) from (1) Los Angeles and (7) Azusa, Los Angeles County.

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1936. A Check-list and Host-index of the Genus *Haemoproteus*. Jour. Parasit., 22: 88–105.

Uegaki, J. 1930. Untersuchungen über die Blutprotozoen von Vögeln der Südsee. Arch. f. Protistenkunde, 72: 74–90.

Wenyon, C. M. 1926. Protozoology: a Manual for Medical Men, Veterinarians and Zoologists (New York, Wm. Wood and Co.), 2 vols., 1563 pp., pls. 1–20, 565 figs. in text.

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A CATALOG AND HOST–INDEX OF THE GENUS *LEUCOCYTOZOON**

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As in the writer's earlier catalogs on blood-inhabiting protozoa a sincere effort has been made to examine the original literature but in some cases, as indicated, it has been necessary to accept the records of other workers. It is hoped that they had access to the original species descriptions or host lists.

Species originally assigned to the genus *Leucocytozoon,* but which for good reason belong to other genera, have been retained in the catalog and footnotes are appended to show, what is thought to be, their correct generic status. However, hosts other than birds have been omitted from the host-list since representatives of this genus are limited to the class Aves.

Certain earlier workers reported "Leucocytozoon stages" in the life cycle of trypanosomes of certain hosts and these, when detected, have been included in the host-list and designated by (t). No decisions are advanced concerning synonymy. Correspondence concerning omissions or errors is invited.

GENUS *LEUCOCYTOZOON* (LEUCOCYTOZOAIRE) DANILEWSKY, 1890

Catalog of *Leucocytozoon* Species

1. *L. akodoni* (Carini and Maciel, 1915). (x) In *Akodon fulginosus,* mouse.
2. *L. anatis* Wickware, 1915. (E) In ducks.
3. *L. anellobiae* (Cleland and Johnston, 1911). (R) In *Anellobia chrysoptera,* honey sucker.
4. *L. anseris* Knuth and Magdeburg, 1922. (E & R) In *Anser domesticus,* domestic goose.
5. *L. ardeae* Rodhain, Pons, Vandenbranden and Bequaert, 1913. (R) In *Ardea goliath,* heron.
6. *L. audieri* Laveran and Nattan-Larrier, 1911. (E) In *Haliaetus vocifer,* eagle.
7. *L. berestneffi* Sambon, 1908. (R) In *Pica pica,* magpie.
8. *L. bouffardi* Leger and Blanchard, 1911. (R) In *Hyphantornis melanocephala,* weaver bird.
9. *L. brimonti* Mathis and Leger, 1910. (R) In *Ixus hainanus,* bulbul.
10. *L. cambournaci* Franca, 1912. (R) In *Emberiza cirlus,* bunting.
11. *L. canis* (James, 1905). (x) In dog.
12. *L. caprimulgi* Kerandel, 1913. (E) In *Caprimulgus fossei,* nightjar.
13. *L. caulleryi* Mathis and Leger, 1909. (R) In *Gallus domesticus,* domestic fowl.
14. *L. centropi* Fantham, 1921. (R or ?E) In *Centropus burchelli,* Burchell's coucal.

* The study supported by a grant from the A. A. A. S. through its affiliate the Nebraska Academy of Sciences.

15. *L. circaëti* Sergent and Fabiani, 1922. (E) In *Circaëtus gallicus,* rapace.
16. *L. danilewskyi* (?) Ziemann, 1898. (E) In *Athene noctua,* little owl.
17. *L. dubreuili* Mathis and Leger, 1911. (R) In (?) *Turdus musicus,* song thrush.
18. *L. eurystomi* Kerandel, 1913. (E) In *Eurystomus gularis,* roller.
19. *L. felis domestici* (Patton, 1908). (x) In *Felis domesticus,* domestic cat.
20. *L. francai* Nikitine and Artemenko, 1927. (R or ?E) In *Turdus pilaris,* thrush.
21. *L. francolini* Kerandel, 1913. (E) In *Francolinum bicalcaratus,* francolin.
22. *L. fringillinarum* Woodcock, 1910. (R) In *Fringilla coelebs,* chaffinch.
23. *L. funambuli* (Patton, 1906). (x) In *Sciurus palmarum* (=*Funambulus pennantii*) palm squirrel.
24. *L. gentili* Leger, 1913. (R) In *Fringilla petronia.* (a–1).
25. *L. kerandeli* Mathis and Leger, 1911. (E) In *Francolinus sinensis,* francolin.
26. *L. laverani* Franca, 1912. (R) In *Garrulus glandarius,* jay.
27. *L. laverani* Franchini, 1923. (E) In *Albanella pallida,* falcon.
28. *L. lebaeufi* Mathis and Leger, 1911. (R) In *Ardetta sinensis,* yellow bittern.
29. *L. legeri* Franca, 1912. (R) In *Scolopax rusticola,* woodcock.
30. *L. leporis* (Patton, 1908). (x) In *Lepus nigricollis,* hare.
31. *L. liothricis* (Laveran and Marulla, 1914). (R) In *Liothrix luteus,* babbler.
32. *L. lovati* Seligman and Sambon, 1907. (E) In *Lagopus scoticus,* red grouse.
33. *L. lutzi* Carini, 1920. (E) In *Scops brasiliensis,* owl.
34. *L. macleani* Sambon, 1908. (E) In *Phasianus colchicus,* pheasant.
35. *L. majoris* (Laveran, 1902). (R) In *Parus major,* great tit.
36. *L. marchouxi* Mathis and Leger, 1910. (R) *Turtur humilis,* dove.
37. *L. mansoni* Sambon, 1908. (E) In *Tetrao urogallus,* capercailzie.
38. *L. martini* Mathis and Leger, 1911. (R) In *Pavo cristatus,* peacock.
39. *L. martyi* Commes, 1918. (E) In *Astur badius sphenurus,* hawk.
40. *L. mathisi* Franca, 1912. (E) In *Accipiter nisus,* sparrow hawk.
41. *L. mesnili* Leger and Mathis, 1909. (R) In *Francolinus sinensis,* francolin.
42. *L. mirandae* Franca, 1912. (R) In *Merula merula,* thrush.
43. *L. monardi* Rodhain, 1931. (R) In *Passer griseus,* sparrow.
44. *L. muris* (Balfour, 1906). (x) In *Mus decumanus,* Norway rat.
45. *L. musculi* (Porter, 1908). (x) In *Mus musculus,* white mouse.
46. *L. neavei* (Balfour, 1906). (E) In *Numida ptilorhyncha.* (a–n & w).
47. *L. ranarum* (Carini, 1907). (♯) In *Rana esculenta,* frog.
48. *L. numidae* Kerandel, 1913 (E) (*) In *Numida meleagris,* guinea fowl.
49. *L. piroplasmoides* (Ducloux, 1908). (ø) In the mule.
50. *L. ralli* Galli-Valerio, 1930. (R) In *Rallus aquaticus,* rail.
51. *L. ratti* (Adie, 1906). (x) In *Mus rattus,* rat.
52. *L. roubaudi* Mathis and Leger, 1911. (R) In *Munia topela,* weaver finch.
53. *L. sabrazesi* Mathis and Leger, 1910. (E) In *Gallus domesticus,* domestic fowl.
54. *L. sakharoffi* Sambon, 1908. (R) In *Corvus corax,* raven.
55. *L. schoutedeni* Rodhain, Pons, Vanderbranden and Bequaert, 1913. (R) In *Gallus bankiva,* jungle fowl.
56. *L. schüffneri* Prowazek, 1912. (E) In *Gallus domesticus,* domestic fowl.
57. *L. seabrae* Franca, 1912. (E) In *Chloris chloris,* green finch.
58. *L. simondi* Mathis and Leger, 1910. (E) In *Querquedula crecca,* teal duck.
59. *L. smithi* (Lavern and Lucet, 1905). (E) In *Meleagris gallopavo,* domestic turkey.
60. *L. struthionis* Walker, 1913. (R) In *Struthio camelus,* ostrich.
61. *L. toddi* Sambon, 1907. (E) In *Asturinula monogrammica,* Congo grew hawk. (a–s).
62. *L. vandenbrandeni* Rodhain, 1931. (R) In *Plotus rufus,* cormorant.
63. *L. ziemani* (Lavernan), 1902. (E) In *Athene noctua,* little owl.
64. *L. ziemani* var. *bubonis* Fantham, 1926. (E) In *Bubo maculosus,* owl.

65. *L. zuccarellii* Leger, 1913. (R) In *Corvus corone,* carrion crow.
66. *L. bonase* Clarke, 1935. (‡) (E) In *Bonasa umbellus,* ruffed grouse.
67. *L. chloropsidis* de Mello, 1935. (‡) (E) In *Chloropsis aurifrons davidsoni.*
68. *L. coraciae benghalensis* de Mello, 1935. (‡) (E & R) In *Coracias benghalensis.*

(x) Probably a Hepatozoon (See Wenyon, 1926).
(E) Spindle-shaped (elongate) host cell.
(R) Circular host cell.
(♯) Probably a Haemogregarine.
(ø) Probably a Leishmania.
(*) Genus in doubt.
(a–s) According to Sambon, 1908.
(a–1) According to Leger, M. and A., 1914.
(a–n & w) According to Neave, 1907, and Wenyon, 1908.

HOST-INDEX FOR THE GENUS LEUCOCYTOZOON

Acanthogenys rufigularis—spiny cheeked honey-eater—: Cleland, 1915.
Accipiter nisus Linné—: Mezincescu, 1909*; (40) Franca, 1912; (40) Franchini, 1924.
Acridotheres tritis—: Maya and David, 1912.
Aex galericulata—: Hamerton, 1931.
Alario alario—alario finch—: Plimmer, 1916; Scott, 1926.
Albanella pallida—: (27) Franchini, 1923.
Alectoris chukar—: Hamerton, 1929.
Alectoris graeca—: Hamerton, 1929.
Ammoperdix heyi—: Scott, 1927.
Anas boscas—: Franca, 1912†*.
Anellobia chrysoptera Lath.—honey sucker—: (3) Cleland and Johnston, 1911.
Anser domesticus—domestic goose—: (4) Knuth and Magdeburg, 1922.
Anser ferus—: Franca, 1912†.
Ardea atricapilla Afzélius—: Leger, A. and M., 1914.
Ardea goliath—heron—: (5) Rodhain, *et al.,* 1913a.
Ardea purpurea—purple heron—: ? Wülker, 1919; Franchini, 1924.
Ardetta sinensis Gm.—yellow bittern—: (28) Mathis and Leger, 1911.
Artamus superciliosus—white-eyebrowed wood-swallow—: Plimmer, 1916.
Asio accipitrinus—: (63) Leger, 1917.
Asio otus Linné (=*Otus sylvestris*)—long-eared owl—: Danilewsky, 1889; Böing, 1925; Hamerton, 1934.
Astur badius—: Hamerton, 1932.
Astur badius sphenurus—: (39) Commes, 1918.
Astur palumbarius—goshawk—: Wasielewski, 1908*.
Astur polyzonoides—little banded goshawk—: Fantham, 1926.
Asturinula monogrammica—congo grew hawk—: (61) Sambon, 1907; Dutton, *et al.,* 1907; Rodhain, *et al.,* 1913.
Asturinula monogrammica meridionalis Hartlaub—Hawk—: (61) Aubert and Heckenroth, 1911.
Athene noctua—little owl—: Danilewsky, 1890*; (16?) Zieman, 1898; (63) Laveran, 1902; Schaudinn, 1904*; Sergents, 1907; Minchin and Woodcock, 1911; Moldovan, 1914; Plimmer, 1914.
Athene noctua bactriana—: Scott, 1928.
Athene sp.—owl—: (16) Berestneff, 1904.
Balearica regulorum—: Rodhain, *et al.,* 1913.
Balaeniceps rex —: Rodhain, *et al.,* 1913.
Bécasse (? *Gallinago media*—snipe)—: Mathis and Leger, 1911b.
Bittern (? *Botaurus stellaris*)—: Wülker, 1919.
Blackbird—: Wülker, 1919.
Bonasa umbellus—ruffed grouse—: (66) Clarke, 1935.

Botaurus stellaris—: see bittern.
Bubo bubo sibiricus—: Böing, 1925.
Bubo lacteus Temminck—: Leger A. and M., 1914.
Bubo maculosus—spotted eagle owl—: (64) Fantham, 1926.
Bubo v. virginianus (Gmelin)—hoot owl—: Coatney and Roudabush, 1937.
Bucorax abyssinicus—: Scott, 1927.
Bustard, dwarf (? *Tetrax tetrax*)—: Wülker, 1919.
Buteo borealis (Gmelin)—red-tailed hawk—: Coatney and Roudabush, 1937.
Butorides atricapilla—: Rodhain, *et al.*, 1913.
Butorides v. virescens (Linné)—green heron—: Coatney, 1937.
Bycanistes sharpei duboisi—: (63?) Schwetz and Fornara, 1929.
Caccabis rufa (=*Perdix rubra*)—red-legged partridge—: Schaudinn, in Prowazek, 1911.*
Canachites canadensis—spruce grouse—: Clarke, 1935a.
Caprimulgus europaeus—: Böing, 1925.
Caprimulgus fossei—night jar—: Kerandel, 1909; (12) Kerandel, 1913.
Carduelis spinus—siskin—: Betham, 1915?
Carine noctua bactriana—: Hamerton, 1930.
Carpodacus pupureus—: Scott, 1927.
Centropus burchelli—Burchell's coucal—: (14) Fantham, 1921.
Centropus monachus Rüppel—: Leger, A. and M., 1914.
Centropus senegalensis—cuckoo—: Aubert and Heckenroth, 1911.
Centropus sinensis—: Mathis and Leger, 1911a.
Centropus superciliosus—: Rodhain, *et al.*, 1913.
Cerchneis tinnunculus Linné—kestrel—: Böing, 1925.
Chenolopex aegyptiaca—: Minchin, 1911.
Chloris chloris (Linné)—: (57) Franca,† 1912a.
Chloropsis zosterops—: Hamerton, 1930.
Chloropsis aurifrons davidsoni—: (67) de Mello, 1935.
Chotorhea corvina—: Hamerton, 1930.
Chouette—: Lutz and Meyer, 1908.
Circaëtus gallicus (Gmel.)—rapace—: (15) Sergent and Fabiana, 1922.
Circus aeruginosus Linné—: Franchini, 1924; Böing, 1925.
Coccothraustes coccothraustes Linné—hawfinch—: Franchini, 1924; Böing, 1925.
Coccothraustes vulgaris japonicus T. u. S.—: Ogawa, 1911.
Columba grisea—: Scott, 1927.
Columba palumbus Linné—wood pigeon—: Böing, 1925.
Colymbus stellatus—: Hamerton, 1932.
Coracias abyssinicus Boddaert—: Leger, A. and M., 1914.
Coracias benghalensis—: (68) de Mello, 1935.
Coracias garrulus—: see roller.
Corcorax melanorhamphus—crow—: (3) (t) Cleland and Johnston, 1911.
Cornivella corvina Shaw—shrike—: Leger, A. and M., 1914.
Corvus corax—raven—: (see also raven) (54) Sambon, 1908; (54) Mathis and Leger, 1911a; (54) Leger, 1917.
Corvus corone—carrion crow—: (65) Leger, 1913; Plimmer, 1917.
Corvus (? *frugilegus*)—rook—: Sakharoff, 1893; Wasielewski, 1908*; Cardamatis, 1911*.
Corvus macrorhynchus japanensis Bp.—: Ogawa, 1911.
Corvus sp.(?)—crow—: Berestneff, 1904.
Coturnix coromandelica—: Scott, 1927.
Cranorhinus corrugatus—: Hamerton, 1932.
Crow—: (16) Berestneff, 1904.
Crypsirhina varianus—: Hamerton, 1929.
Ctocampsa flaviventris—: Hamerton, 1929.
Curaeus aterrimus—Chilian starling—: Plimmer, 1915.

Cyanocitta c. cristata (Linné)—blue jay—: Coatney and Roudabush, 1937.
Cyanospiza ciris—nonpareil finch—: Plimmer, 1916.
Dacello gigantea—: Hamerton, 1929.
Diatropura progne—: Hamerton, 1933.
Duck—: (2) Wickware, 1915; (2) Hartman, 1929; (2) O'Roke, 1930, 1931, 1931a, 1934.
Emberiza cirlus Linné—bunting—: (10) Franca, 1912a.
Emberiza variabilis Temm.—: Ogawa, 1911.
Entomyza cyanotus—: (3) (t) Cleland and Johnston, 1911.
Erythrura prasina (Sparrm.)—: Uegaki, 1930.
Estrilda astrild—: Hamerton, 1934.
Estrilda phenicotis—waxbill—: Scott, 1926.
Eunetta falcata—falcated duck—: Plimmer, 1915.
Europtoma afer—: Zupitza, 1909†*; Hamerton, 1929.
Eurystomus gularis—roller—: Kerandel, 1909; (18) Kerandel, 1913.
Falco circaetus—: Rodhain, *et al.*, 1913.
Falco nisus—hawk—: (63) Mezincescu, 1909; Chingaréva, 1911.
Falco peregrinus Tunstall—: Ogawa, 1911; Hamerton, 1931.
Falco tinnunculus—: Sergents, 1904.
Fondia madagascariensis—: Maya and David, 1912; Hamerton, 1932.
Francolinus bicalcaratus Linné—Francolin—: Kerandel, 1909; (21) Kerandel, 1913; (21) Leger A. and M., 1914.
(?)*Francolinus chinensis*—: Mesnil, 1909.
Francolinus (?*chinensis*)—: Mesnil, 1909.
Francolinus hubbardi—francolin—: Ross, 1911*.
Francolinus mulemae—: Minchin, 1911.
Francolinus pondicherianus—: Scott, 1927.
Francolinus sinensis Osbeck—francolin—: (41) Leger and Mathis, 1909; (25) Mathis and Leger, 1911a.
Francolinus sp.—: Wenyon, 1908; Todd and Wolbach, 1912; Rodhain, *et al.*, 1913†.
Fringilla coelebs—chaffinch—: (22) Woodcock, 1910; Schaudinn in Prowazek, 1911*.
Fringilla kawarahiba minor T. u. S.—: Ogawa, 1911.
Fringilla montifringilla—: Scott, 1926.
Fringilla petronia—: (24) Leger, 1913†.
Fuligula marila—scaup duck—: Plimmer, 1912.
Gallinago media—: (see Bécasse).
Gallinula chloropus—moor-hen—: Coles, 1914*.
Gallus bankiva—: (55) Rodhain, *et al.*, 1913a.
Gallus domesticus—domestic fowl—: (53) Mathis and Leger, 1909, 1911a; (13) *Ibid.*, 1910, 1911a; (56) Prowazek, 1912; (genus?) Macfie, 1916.
Gallus ferrugineus Linné—: (53) Mathis and Leger, 1911a.
Gallus gallus—: Scott, 1927; Hamerton, 1929.
Gallus lafayettei—: Hamerton, 1929.
Gallus sonnerati—: Scott, 1927.
Garrulus glandarius Linné—: (see also jay) (26) Franca, 1912; (27) Franchini, 1924; Böing, 1925; Hamerton, 1931.
Garrulus japonicus T. u. S.—: Ogawa, 1911.
Geopelia striata—dove—: Maya and David, 1912.
Geocichla varia (Pall.)—: Ogawa, 1911.
Guttera pucherani Hartb.—: Keysselitz and Mayer, 1909.
Gymnorhina leuconota—: Hamerton, 1930.
Haliaetus vocifer—eagle—: (6) Laveran and Nattan-Larrier, 1911.
Heron, gray—: Wülker, 1919.
Hyphantornis cucullatus—: Leger, A. and M., 1914.
Hyphantornis melanocephala Linné—: (8) Leger and Blanchard, 1911.

Hypsipetus amaurotis (Temm.)—: Ogawa, 1911.
Ibis molucca—: Scott, 1927.
Ixus hainanus—bulbul—: (9) Mathis and Leger, 1910a.
Janthocincla rufigularis—: Scott, 1926.
Jay (? *Garrulus glandarius*)—: Wülker, 1919.
Lagopus scoticus—red grouse—: (32) Seligman and Sambon, 1907; (32) Fantham, 1910.
Lagopus lagopus—Willow ptarmigan—: Allen and Levine, 1935.
Lanius collurio Linné—shrike—: Böing, 1925.
Lanius excubitor Linné—: Böing, 1925.
Liothrix luteus (Scopoli)—babbler—: (31) Laveran and Marullaz, 1914; Hamerton, 1929, 1931.
Lophophurus impeyanus—: Hamerton, 1932.
Lyurus tetrix Linné—: Böing, 1925.
Magpie (? *Pica pica*)—: Sakharoff, 1893; Wülker, 1919.
Megaloprepia magnifica—: Scott, 1927.
Meleagris gallopavo domestica—domestic turkey—: Smith, 1895; (59) Laveran and Lucet, 1905; (59) Volkmar, 1929; Skidmore, 1931, 1932.
Melierax gabar—: Leger and Husnot, 1912.
Melithreptus lunatus—: Hamerton, 1933.
Merula merula Linné—: (42) Franca, 1912a.
Merula migratoria—American robin—: (35?) Novy and MacNeal, 1905.
Milvus aegypticus—Egyptian kite—: (46) Neave, 1909.
Milvus migrans—: Rodhain, *et al.*, 1913.
Milvus milvus Linné—: Böing, 1925.
Monedula monedula—jackdaw—: Chingaréva, 1911.
Monticola sp.—: see thrush.
Montacilla alba—: Franchini, 1924.
Munia topela Swinh.—: (52) Mathis and Leger, 1911.
Myzantha garrula—: (3) (t) Cleland and Johnston, 1911.
Myzomela sanguineolenta—: (genus?) Cleland and Johnston, 1911; Cleland, 1915.
Nucifraga caryocatactes—: Scott, 1927.
Numida meleagris Linné—Guinea fowl—: (46?) Kerandel, 1909; (46?) (48?) Kerandel, 1913; Franchini, 1924; Leger, A. and M., 1914.
Numida ptilorhyncha—guinea fowl—: (46) Balfour, 1906*; (46) Wenyon, 1908; (46) Neave, 1907; Minchin, 1911†; (46) Rodhain, *et al.*, 1913.
Nycticorax nycticorax Linné—black-crowned night heron—: Aubert and Heckenroth, 1911; Franchini, 1924.
Nycticorax nycticorax hoactli (Gmelin.)—black-crowned night heron—: Coatney, 1937.
Otus scops—: Hamerton, 1931.
Otusbakkamoena glablipes (Swinhoe)—: Ogawa and Uegaki, 1927.
Otus brachyotus—short-eared owl—: Schaudinn in Prowazek, 1911*.
Otus sylvestris—: see *Asio otus*.
Oreicola ferra—chat—: Plimmer, 1914.
Oriolus galbula—golden oriole—: Cardamatis, 1911.
Oriolus viridis—: (3) (t) Cleland and Johnston, 1911.
Oryzornis oryzivora—: Uegaki, 1930.
Owl (small white)—: (16?) Zieman, 1898 (a–s).
Pardalotus melanocephalus—: (genus?) Cleland and Johnston, 1911.
Partridge (francolin partridge)—: (46?) Wenyon, 1908.
Parus major—: (see also tit) (35) Laveran, 1902; (35) Franca, 1912a; Franchini, 1924.
Passer chloris—: Franca, 1912†.
Passer domesticus—sparrow—: Ruge, 1901*; Maya and David, 1912.
Passer griseus—sparrow—: (43) Rodhain, 1931.
Passer (? *montanus*)—sparrow—: Mine, 1914.

Pavo cristatus Linné—peacock—: (38) Mathis and Leger, 1911.
Pedioecetes phasianellus—sharp-tailed grouse—: Saunders, 1935.
Perdix perdix (Linné)—: Böing, 1925.
Perdix rubra Brisson—red partridge—: (63) Sergent, 1917.
Phaenicurus frontalis—: Hamerton, 1934.
Phasianus colchicus Linné—pheasant—: (34) Sambon, 1908; Böing, 1925.
Pica melanoleuca—: (7) Leger, 1917.
Pica pica—: (see also magpie) Berestneff, 1904; (7) Sambon, 1908; Böing, 1925.
Piranga flava (Vieill.)—: Mazza and Fiora, 1930.
Pisorhina choliba Vieill.—: see *Scops brasilensis*.
Pishorina scops—: Franchini, 1924.
Pitangus sulphuratus bolivianus (Lefr.)—: Mazza and Fiora, 1930.
Plesiositagra nigriceps—: Hamerton, 1933.
Plotus rufus—cormorant—: (62) Rodhain, 1931.
Podargus cuvieri—: Scott, 1927.
Podargus strigoides—: Cleland, 1915.
Prionops plumatus Shaw—: Leger, A. and M., 1914.
Prionops talacoma—: Rodhain, *et al.*, 1913.
Propasser rhodochrous—rose finch—: Plimmer, 1917.
Pternistes infuscatus—bare-throated francolin—: Ross, 1911*.
Ptilogenys cinereus—: Scott, 1928.
Ptilonorhynchus violaceus—: Scott, 1926.
Ptilolis fusca—: (3) (t) Cleland and Johnston, 1911; Cleland, 1915.
Ptilotis penicillatus—: Hamerton, 1933.
Pycnonotus barbatus Desfontaines—bulbul—: Leger, A. and M., 1914.
Pycnonotus sp.—bulbul—: Maya and David, 1912.
Pyromelaena flammiceps Swainson—: Leger, A. and M., 1914.
Pyromelana franciscana—: Hamerton, 1930, 1931, 1933.
Pyrrhula pyrrhula—bullfinch—: Scott, 1927; Hamerton, 1929, 1930.
Pyrruha griseiventris—: Hamerton, 1930.
Querquedula crecca Linné—: (58) Mathis and Leger, 1910.
Rallus aquaticus—: (50) Galli-Valerio, 1930.
Raven (? *Corvus corax*)—: Sakharoff, 1893.
roller (? *Coracias garrulas*)—: Wülker, 1919.
Rook (? *Trypanocorax frugilegus*)—: Sakharoff, 1893.
Ruticilla aurorea (Gmel.)—: Ogawa, 1911.
Schizorhis leucogaster—: Scott, 1927.
Scolopax rusticola Linné—: Ogawa, 1911; Mathis and Leger, 1911; (29) Franca, 1912; Scott, 1927.
Scops brasiliensis (= *Pisorhina chloiba* Vieill.)—owl—: (33) Carini, 1920.
Scops capensis—: (16?) Rodhain, *et al.*, 1913.
Scops giu—Scops owl—: (16) Sergents, 1902†; Plimmer, 1913, 1917.
Scops semitorques T. u. S.—: Ogawa, 1911.
Scotiaptex lapponica—: Scott, 1927.
Scotopelia peli—: (16?) Rodhain, *et al.*, 1913.
Serinus canarius—wild canary—: Scott, 1926, 1928.
Sialia s. sialis—bluebird—: Huff, 1935.
Sittagra monaca—: Rodhain, *et al.*, 1913.
Spatula clypeata—: Hamerton, 1931.
Spinus citrinellus corsicanus—: Scott, 1928.
Spreo superbus—: Scott, 1926.
Strix flammea trimaculata—barn owl—: Kerandel, 1909; (16) Kerandel, 1913.
Strix otus—long-eared owl—: Wasielewski, 1902*, 1908*.
Strix sp.—: Danilewsky, 1889, 1894.
Struthio camelus—ostrich—: (60) Walker, 1913.
Sturnus vulgaris—common starling—: Coles, 1914*.
Suthora gularis—: Hamerton, 1931.

Sycalis arvensis—: Scott, 1926.
Sycobrotus strictifrons—: Hamerton, 1930.
Sylvia curruca—: Scott, 1926.
Syrnium aluco—tawny owl—: Danilewsky, 1889; (16) Laveran, 1903; (63) Sergents, 1907; Mayer, 1911; (63?) Cardamatis, 1911; Plimmer, 1914, 1915.
Syrnium noctua—: Sergents, 1904.
Tetrax tetrax—: see bustard.
Tetrao urogallus Linné—capercailzie—: (37) Sambon, 1908; Böing, 1925.
Thrush (? *Monticola saxatilis*)—: Wülker, 1919.
Tit, great (? *Parus major*)—: Wülker, 1919.
Totanus ocropus—: Franchini, 1924.
Tragopan blythi—: Hamerton, 1934.
Tragopan satyra—: Scott, 1927; Hamerton, 1932, 1934.
Tropidorhynchus corniculatus—honey sucker—: Brienl, 1912?.
Trypanocorax frugilegus—: see rook.
Turdus fuscatus Pall—: Ogawa, 1911.
Turdus musicus Linné—: Franca, 1912a; (17) Mathis and Leger, 1911a; Coles, 1914*.
Turdus obscurus Gmel.—: Ogawa, 1911.
Turdus pallidus Gmel.—: Ogawa, 1911.
Turdus pilaris—: (2) Nikitine and Artemenko, 1927.
Turtur auritus—: Leger, M., 1913.
Turtur humilis Bp. (36) Mathis and Leger, 1910.
Turtur orientalis (Latham)—: Ogawa, 1911.
Turtur semitorquata—: Minchin, 1911†.
Turtur senegalensis Linné—: Leger, A. and M., 1914.
Turtur turtur—: Franchini, 1924: Scott, 1927.
Vanellus vanellus Linné—: Böing, 1925.
Vultur sp.—: Chingaréva, 1911.
Zosterops chlorophea—: Maya and David, 1912.
Zosterops japonica T.u.S.—: Ogawa, 1911.
Yunx torquilla—: Franchini, 1924.

(†) According to Leger, M. and A., 1914.
(*) According to Wenyon, 1926.

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RESEARCH NOTES

A QUESTION OF PRIORITY IN THE USE OF THE NAME *EIMERIA MUSTELAE*

Dr. A. A. Kingscote published in the Journal of Parasitology (1934, 20: 252–253) a note entitled "*Eimeria mustelae* n.sp. from *Mustela vison*." This author stated that he examined for enteric coccidiosis carcasses and feces of mink which were obtained from ranches in Canada and the United States. The oöcysts were oval and egg-shaped; wall double; yellowish-brown; inner layer thicker than colorless external membrane; no micropyle; size ranges from 17.0–22.1 μ × 9.0–18.0 μ, average of 100 oöcysts 20.3 μ × 14.6 μ. The author named this coccidia *Eimeria mustelae*. My pupil Miss P. S. Iwanoff-Govzem published in the same year, 1934, in the "Deutsche tierärztliche Wochenschrifft" an article entitled "Zum Vorkommen von Coccidien bei kleinen wilden Säugetieren." In this publication she was describing the *Eimeria* in three weasels (*Mustelae nivalis*) found in Kasakhstan (Middle-Asia). The animals had a hemorrhagic inflammation of the colon and the feces showed a multitude of oöcysts. They were round or oval with a thick membrane; no micropyle; size of oval oöcysts 17.6–26.4 μ × 15.4–24.2 μ average 21.6 μ × 18.8 μ; the ratio of length to breadth was 1 : 0.8–0.96, average 1 : 0.88. Size of the round oöcysts was 17.6–25.2 μ, average 20.2 μ. The oöcysts when cultivated gave in 72 hours four spores with two sporozoites in each of them. No residual bodies either in the oöcysts or in sporozoites. The author named this species *Eimeria mustelae*. Thus we may see that both authors gave the same name of *Eimeria mustelae* to two parasites entirely dissimilar because the hosts of both belonged to the genus *Mustela*. Since the article of Miss P. S. Iwanoff-Gobzem was published on March 19, 1934, and Dr. A. A. Kingscote's note appeared in June of the same year the first author has priority in the use of the name *Eimeria mustelae*. Dr. Kingscote should choose another name for the species he found in *Mustela vison*.—W. L. Yakimoff, *Leningrad, U.S.S.R.*

THE INTESTINAL PARASITES OBSERVED IN FECAL SAMPLES FROM 729 COLLEGE FRESHMEN

For the past three years this department has conducted a series of surveys for the purpose of determining the incidence of intestinal parasites of man for the state. So far attention has been given to the local urban population and to the incoming freshman classes of the University of Georgia at Athens. The results of two of these surveys have been reported elsewhere by the author (1936, Amer. Jour. Trop. Med., 16: 39–45, and 1936a, Jour. Parasit., 22: 301–302). The present paper reports the observations made since our last report on fecal samples from 729 freshmen students from two of the units in the University System of Georgia.

Stools were collected during the week of the physical examination of students (September 17th–24th, 1936). These specimens were placed in 2 oz. tin containers and preserved with a 6 per cent solution of formalin for future diagnoses. At the time of the examination the contents of each container was thoroughly stirred, filtered through a double layer of gauze into a centrifuge tube, and concentrated by centrifugation. Examinations were made from an iodine-stained smear of the concentrate. In all cases where it was impossible to make an accurate diagnosis from the preserved specimen a fresh stool was requested for re-examination. In the results recorded for the incidence of the various parasites encountered in this study no attempt is made to indicate the total number of individual stools examined in order to determine the presence or absence of the parasite in question. No stool was more than two months old at the time the examination was made.

Results.—The incidence of the various parasites encountered in the 729 individuals examined is given according to the number of times they occurred, giving the total number of cases for each parasite and their percentages as follows: Negative stools, 331 (45.4%); stools positive for one or more parasites, 398 (54.6%); *Endamoeba histolytica,* 38 (5.2%); *Endamoeba coli,* 179 (24.5%); *Blastocystis hominis,* 169 (23.2%); *Endolimax nana,* 146 (20.0%); *Giardia lamblia,* 61 (8.4%); *Iodamoeba williamsi,* 19 (2.6%); *Chilomastix mesnili,* 5 (0.7%); *Dientamoeba fragilis,* 10 (1.4%); *Trichomonas hominis,* 2 (0.3%); *Necator americanus,* 22 (3.0%); *Trichocephalus trichiurus,* 2 (0.3%) *Ascaris lumbricoides,* 1 (0.1%); *Enterobius vermicularis,* 1 (0.1%); *Strongyloides stercoralis,* 1 (0.1%); *Hymenolepis nana,* 2 (0.3%).

Of the 729 individuals examined 360 (49.4%) were males and 369 (50.6%) were females. The incidence of infection for the various parasites encountered in the individuals of these two groups is outlined in Table I. Attention is called

TABLE I. *The distribution of intestinal parasites in male and female students*

Infection	Males		Females	
	Number	Per cent	Number	Per cent
Negative stools	148	41.1	183	49.6
Positive stools	212	58.9	186	50.4
Total	360	100.0	369	100.0
Endamoeba histolytica	24	6.7	14	3.8
Endamoeba coli	85	23.5	94	25.5
Endolimax nana	85	23.5	61	16.5
Iodamoeba williamsi	12	3.3	7	1.9
Dientamoeba fragilis	3	0.8	7	1.9
Giardia lamblia	32	8.9	29	7.9
Chilomastix mesnili	4	1.1	1	0.3
Trichomonas hominis	2	0.6		
Blastocystis hominis	93	25.8	66	17.9
Hymenolepis nana	2	0.6		
Necator americanus	21	5.8	1	0.3
Strongyloides stercoralis	1	0.3		
Ascaris lumbricoides	1	0.3		
Enterobius vermicularis	1	0.3		
Trichocephalus trichiurus			2	0.5

to the fact that the various species of protozoa encountered are more generally met with in the males than in the females. The two exceptions are to be found in the case of *Endamoeba coli* and *Dientamoeba fragilis,* both of which occurred more frequently among the females. With the exception of a single case of *Necator americanus* and the two cases of *Trichocephalus trichiurus* all helminths encountered in the study occurred among the males.

The examination of the 398 individuals showing one or more parasites reveals the following intensity of infections: Individuals having a single infection, 218 (54.8%); cases showing two infections, 124 (31.2%); cases with three infections, 46 (11.5%); cases harboring four infections, 6 (1.5%); cases having five infections, 3 (0.8%); and cases harboring six infections, 1 (0.3%). The majority of the cases showing the larger numbers of parasitic species per case occurred among the males. In the female group at least one-half of the total number of infected individuals harbored only a single parasite. Only two cases occurred among the females in which more than three parasites were recorded.

The geographic distribution of the 729 individuals included in this survey are arranged according to the states from which they are registered at the University as follows: Alabama, 3; Arkansas, 1; Connecticut, 3; Florida, 14; Georgia, 658; Illinois, 1; Kentucky, 1; Louisiana, 1; Maryland, 1; Massachusetts, 2; Michigan, 2; New Jersey, 3; New York, 8; North Carolina, 3; Ohio, 2; Pennsylvania, 6; South Carolina, 7; Tennessee, 5; Texas, 1; and Virginia, 5. The geographic distribution of the various pathogenic organisms encountered in the individuals coming from these various sections of the country may be summarised as follows:

Endamoeba histolytica: Georgia, 34 cases; Alabama, Ohio, Tennessee, and Virginia, 1 case each: *Giardia lamblia:* Georgia, 55 cases; Tennessee, 2 cases; Alabama, Florida, Michigan, and Ohio, 1 case each: *Trichomonas hominis:* Georgia and Tennessee, 1 case each: *Necator americanus:* Georgia, 22 cases: *Ascaris lumbricoides:* Georgia, 1 case: *Strongyloides stercoralis:* Georgia, 1 case: *Enterobius vermicularis:* Georgia, 1 case: *Trichocephalus trichiurus:* Georgia, 1 case: *Hymenolepis nana:* Georgia, 2 cases. It might be pointed out that *Dientamoeba fragilis* has been omitted from the above list of pathogenic organisms. The presence of this parasite in 10 (1.4%) of the individuals included in this survey is significant.—Elon E. Byrd, *Zoology Department, University of Georgia.*

A NEW *DIPLOSTOMULUM* FROM CHINA

A new species of *Diplostomulum* has been found to be abundant in a number of vertebrates at Canton and, though the life history has not yet been elucidated, certain points in regard to its biology appear to justify a preliminary report.

Diplostomulum mutadomum n.sp. (Figs. 1 and 2)

Description.—Diplostomulum. Living worm very active, .350–1.000 mm. long by .175–.325 mm. wide, depending on degree of contraction. Forebody elongate oval, slightly concave with free margin continuing ventrally across posterior end. Conical hindbody, which changes little in shape, .065–.075 mm. long by .050–.060 mm. wide at base; often not visible. Anterior part covered thickly with spines 2.5 μ long which thin out posteriorly and are not found posterior to middle of

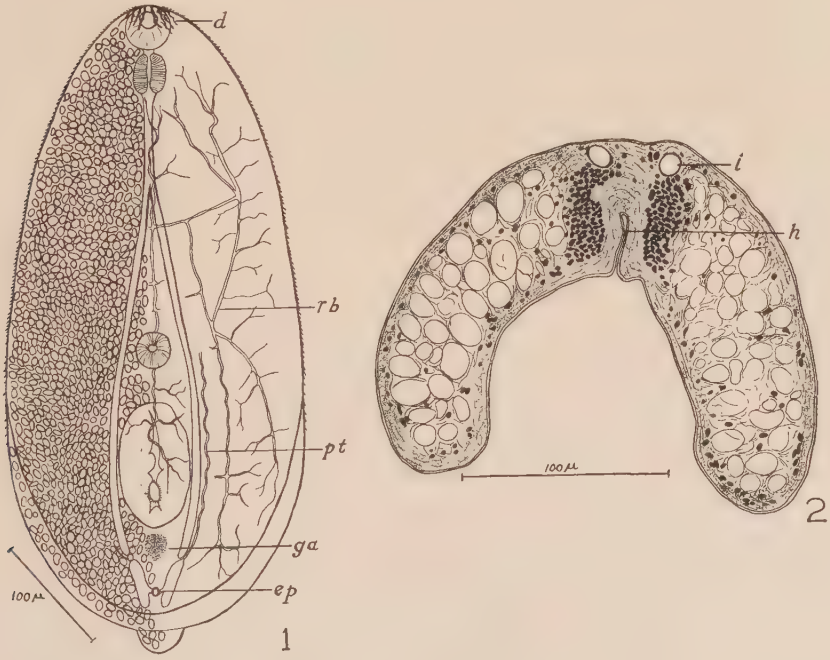


Fig. 1 *Diplostomulum mutadomum,* ventral view. Calcareous granules of reserve bladder omitted on right to show principal vessels as seen under compression. d, oral ducts; ep, excretory pore; ga, genital anlage; pt, tubule of primary excretory system; rb, vessels of reserve bladder.

Fig. 2 Transverse section through holdfast organ of *Diplostomulum mutadomum.* h, holdfast; i, intestinal cecum.

holdfast organ. Oral sucker terminal, with weakly developed musculature, 43–47 μ long by 48–54 μ wide. Terminations of eight ducts can be seen with difficulty on dorsal side of oral sucker. Acetabulum posterior to middle of body in extended specimens; 39–44 μ long by 37–49 μ wide. Holdfast organ separated from hind margin of acetabulum by about width of acetabulum; 121–130 μ long by 73–78 μ wide; external opening a small oval; 20–30 μ long, which opens dorsally into a long slit-like cavity about 100μ long surrounded by glandular cells. No trace of lateral suckers or ear-like appendages near oral sucker. Prepharynx very short. Pharynx 46–50 μ long by 38–42 μ wide. Oesophagus long and straight forking a little less than half way from pharynx to acetabulum at an acute angle into the intestinal ceca which extend to lateral horns of excretory bladder. A small group of deeply staining cells between ends of ceca appear to be genital anlage. Excretory pore on dorsal side of hindbody almost at posterior end and connected by short tube to middle of cross bar of H-shaped excretory bladder. From anterior end of each horn of bladder, a collecting tubule passes anteriorly, lateral to ceca. At level of anterior end of holdfast, collecting tubule branches into smaller divisions which cannot be traced. Reserve excretory bladder a branching system of vessels containing calcareous granules in terminal vesicles. Granules completely fill body and conceal primary excretory system; vessels and vesicles of reserve bladder visible only when worm is compressed. Spherical granules 7–10 μ in diameter; larger ellipsoid granules 14–25 μ long appear to result from fusion of smaller spherical granules. Principal vessels of reserve system are as follows: two longitudinal vessels on each side in posterior half of body unite at level of acetabulum but separate again into two anterior branches; a short distance anterior to acetabulum a transverse vessel connects the four longitudinal vessels; lateral longitudinal vessels continue anteriorly from transverse vessel; median longitudinal vessel extends from posterior edge of acetabulum nearly to oral sucker. Worms enclosed in connective tissue capsules .300–.650 mm. long with walls 30–50 μ thick. No cyst of parasitic origin.

Hosts.—Natrix piscator (Schneider), *N. stolata* (L.), *Enhydris chinensis* (Gray), *Elaphe radiata* Schlegel, *Eumeces chinensis* (Gray), *Rana limnocharis* Gravenhorst, *R. guentheri* Boulenger, *R. rugulosa* Wiegmann, and *Microhyla pulchra* Hallowell. (Experimental hosts: white rat, chick, duckling, and short-eared owl.)

Location.—Muscles.

Distribution.—Canton, China.

Type specimen.—Department of Biology, Lingnan University.

A few specimens of the snakes: *Enhydris plumbea* (Boie), *Ptyas korros* (Schlegel), *Ptyas mucosus* (L.), and *Naja naja atra* (Cantor); and the frogs: *Polypedates leucomystax megacephalus* Hallowell, *Microhyla ornata* (Dumeril et Bibron), *Kaloula pulchra* Gray, and *Kalophrynus pleurostigma interlineatus* Blyth were uninfected. In the snake hosts the parasites are usually found in the muscles between the ribs or just below the vertebrae in the anterior part of the body where they may be present in great numbers. Even in very heavily infected specimens they are seldom found in the posterior part of the body. A few are usually found in the mesenteries. In frogs the parasites are usually found in the muscles of the legs.

No differences in size or structure have been detected between the diplostomula from these different hosts. The similarity in structure between the larvae from frogs and snakes, together with the fact that those snakes which are heavily infected live principally on frogs while the lightly infected species do not, suggests, though it has not been experimentally demonstrated, that snakes become infected from eating frogs. Larvae from either frogs or snakes, when fed to white rats, migrate into the diaphragm and intercostal muscles and become encapsulated without any change in structure. Twenty-four hours after infection the larvae were found in the intestinal wall, mesenteries, diaphragm, and thoracic cavity. In animals examined after longer periods diplostomula were found only in the

diaphragm and intercostal muscles, where they have been found as long as 290 days after infection. In one case about 50 larvae from the muscles of two experimentally infected rats were fed to a third rat from whose intercostal muscles 7 larvae were recovered 38 days later. Larvae from snakes were fed to chicks, ducklings, and one short-eared owl, *Asio flammeus flammeus* Pontoppidan, and in each case some of the larvae were found in the muscles at autopsy. The possibility of previous infection of the owl is not excluded, although another owl from the same source harbored no strigeid larvae; but whatever the source of the infection, the fact remains that diplostomula morphologically indistinguishable from those of local reptiles and amphibians have been found in the muscles of this bird. Dr. H. T. Chen of this department has found a number of larvae which appear to be *D. mutadomum* in local shrews, *Suncus coerulus*. Two attempts to infect cats have given negative results. No evidence has yet appeared as to the existence of a mesocercaria stage as described by Bosma (1934, Trans. Am. Micro. Soc., 53: 116–153) for *Alaria mustelae* which the present species resembles in some respects.

Hughes (1929, Occ. Papers Mus. Zool. Mich. 202) lists 6 species of the larval group *Diplostomulum* which lack the lateral sucking cups characteristic of the majority of the group. From these *D. mutadomum* appears to be sufficiently distinct to be described as a new species. In *D. putorii* (von Linstow, 1877, Arch. f. Naturg., 43: 191) from the gut and oesophagus of *Foetorius putorius,* the pharynx and acetabulum are larger than the oral sucker. In *D. nanus* (Stiles and Goldberger, 1908, Hyg. Lab. Bull. 40) from the African partridge, *Francolinus subtorquatus,* the pharynx is small and poorly defined, the oesophagus is short, and the intestinal ceca are greatly dilated. No spines were found. *D. mutadomum* differs from *D. sirtale* (Nicoll, 1912, Proc. Zool. Soc., 767–770) from the snake, *Tropidonotus sirtalis,* in that the acetabulum is posterior rather than anterior to the middle of the body, the holdfast organ is much larger than the acetabulum, and the excretory bladder is H-shaped rather than triangular. *D. mutadomum* is considerably larger than *D. vegrandis* (La Rue, 1917, Occ. Papers Mus. Zool. Mich. 35) from *Thamnophis marciana* and *T. eques* and differs in the shape of the excretory bladder. Furthermore neither *D. sirtale* nor *D. vegrandis* is said to be spined and both are found in the mesenteric fat bodies of the snake hosts. In *D. odhneri* (Travassos, 1924, C. R. Soc. Biol., 91: 835–838) from the subcutaneous tissue of the fish, *Haemulon* sp., the oral sucker is larger and the pharynx smaller than the acetabulum, and the long, slender intestinal rami reach almost to the posterior extremity of the body. Spines are not mentioned as being present on the cuticle, and the genital anlage could not be seen. *D. joyeuxi* Hughes from *Rana esculenta ridibunda* in Africa has well-developed reproductive organs which differentiate it from the present species as seen in frogs. Bosma mentions an undescribed *diplostomolum* without lateral sucking cups in the muscles of the mink.

It appears that the elongate diplostomula without lateral suckers which are found principally in amphibia, reptiles, birds, and mammals form a clearly defined group in which specific differences are slight. The experiments reported here indicate that host relationships cannot be depended upon for differentiation of species and it is improbable that a satisfactory classification can be attained without knowledge of the adult stages. Further experiments with cats, carnivorous birds, and other animals are being undertaken in an attempt to learn the entire life history. The author wishes to express his gratitude to Dr. O. W. Olsen for aid in checking certain literature.—F. G. Wallace, *Dept. of Biology, Lingnan University, Canton, China.*

THE LIFE HISTORY OF *CREPIDOSTOMUM CORNUTUM* (OSBORN)

In the summer of 1930 a large collection of *Sphaerium* sp. from a stream near Dixboro, Michigan yielded specimens infected with a species of ophthalmoxiphidiocercaria. They resembled the young metacercariae of *Crepidostomum cornutum* found in the cardiac region of the crayfishes of this locality; therefore, parasite-free crayfishes, *Cambarus immunis,* were exposed to them. Within two hours after exposure, unencysted cercariae were found in the cardiac region of several crayfishes examined. Crayfishes examined 24 hours later yielded newly encysted metacercariae. A brief study of the life history was made at this time but other work prevented its completion. In the meantime, Hopkins (1934), Ill. Biol. Mono., 13 (2): 1–80, published a monograph on the papillose Allocreadiidae which includes nine members of the genus *Crepidostomum.* Of these he described in full only the life history of *C. cooperi.* It is the sole life history of the genus established experimentally. Hopkins described the adult and metacercaria of *C. cornutum* but reported the remainder of the life cycle as unknown. Though the life history of *C. cornutum* in most respects parallels that of *C. cooperi,* the following data are now made available as a further contribution to the knowledge of the genus.

The adult and metacercaria of *C. cornutum* are well described by Hopkins. Old metacercariae produce considerable numbers of apparently well-formed eggs but I twice attempted unsuccessfully to incubate eggs laid within the cysts. This experiment indicates that the eggs produced by the metacercariae are not fertilized. A few eggs were collected from worms removed from the intestines of rock bass, *Ambloplites rupestris,* and large-mouth black bass, *Aplites salmoides.* They were in the single cell stage when first laid. Miracidia hatched from these eggs in about two weeks with the incubator temperature at 36° C. The small number of eggs precluded a detailed study of the miracidium. Those measured within the eggs were about 0.060 by 0.300 mm. The miracidium possesses a large more or less centrally located eyespot which becomes evident within the first week of development. A short gut and a pair of flame cells could be identified in the active miracidium.

Redia.—Incidence of infection in several thousand *Sphaerium* collected in streams around Ann Arbor ranged from 13 to 49 per cent. Often cercaria *C. cornutum* was the only species present. Very young rediae occur in gills and mantle, maturing and mature in the liver. Young rediae (Fig. 1) are motile and possess a slender, elongate gut which often appears as several strings of fairly large cells confined to the anterior half of the body. Three pairs of unicellular glands resembling penetration glands lie adjacent to the pharynx; their ducts open anterior to it. Hopkins found some young rediae of *C. cooperi* with eyespots. He suggested the probability that they had developed directly from the miracidia but this is to be doubted in the light of the existing knowledge of the development of rediae.

Fully mature rediae are elongate and non-motile; gut and gland cells are absent and frequently even the pharynx is not discernible. A birth pore is absent. Old rediae may thus resemble elongate sporocysts. Some rediae contained as many as 30 to 40 active cercariae as well as a few germ balls. Ten rediae containing cercariae and killed in hot 10 per cent formalin measured 0.820–1.900 mm. by 0.250–0.380 mm. (av., 0.995 by 0.321 mm.). The smallest pharynx measured 0.024 by 0.024 mm., the largest 0.029 by 0.034 mm. (av., 0.025 by 0.031 mm.). Pharynges of the young rediae measured practically the same as stated for old rediae.

Cercaria.—The cercaria (Fig. 2) is fully formed within the redia. Recently emerged cercariae are extremely active. As they swim, the long slender tail describes an arc about the body and often it is in advance of it. Recently emerged cercariae are positively phototropic but this reaction gradually becomes strongly negative.

The body is transparent. Both suckers are provided with sticky protuberances

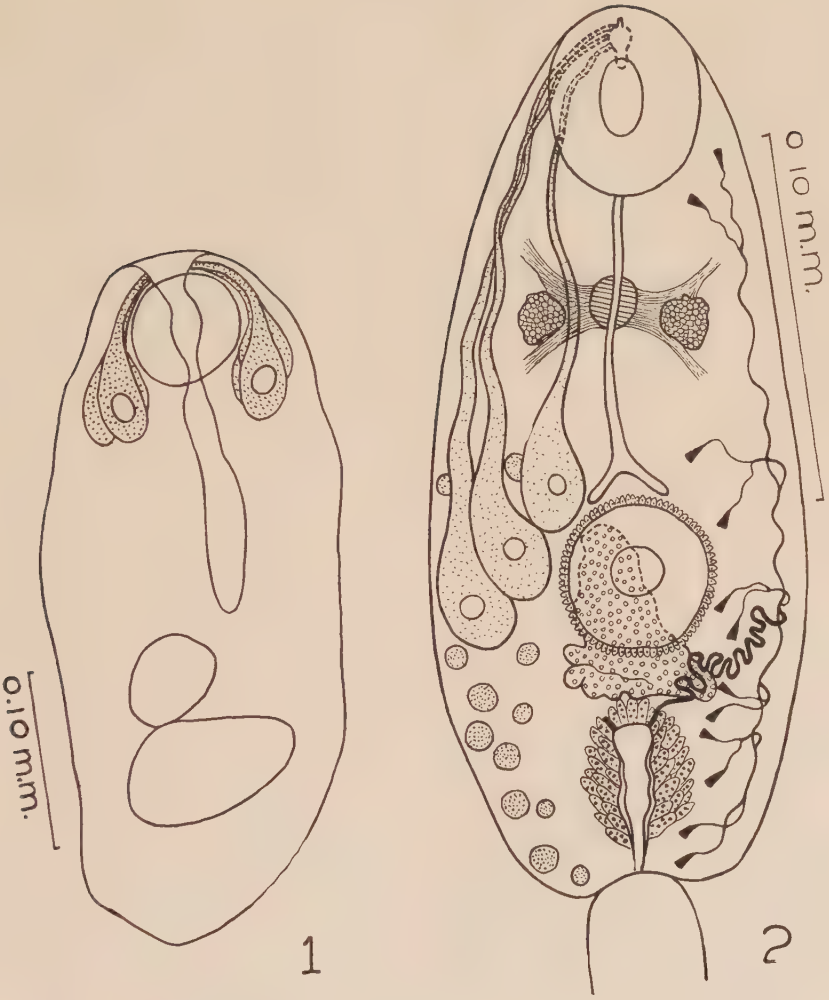


FIG. 1. A young redia of *C. cornutum*. FIG. 2. A cercaria of *C. cornutum*.

of the same nature as those described in cercaria *C. cooperi* by Hopkins. They are difficult to observe in the oral sucker but are especially prominent as a fringe around the edge of the fully extended or flattened ventral sucker. The oral sucker is somewhat larger than the ventral. Three large pear-shaped penetration glands are located on each side of the ventral sucker. The ducts of the two outer pairs run forward laterally; those of the inner pair are medial. They penetrate the oral sucker and open on each side of the stylet located in the anterodorsal portion of the sucker. The contents of the penetration glands are finely granular and stain a faint yellow with neutral red.

The prepharynx is long; the pharynx of moderate size, located about midway between the oral and ventral suckers. The esophagus is generally inconspicuous, often apparently lacking. It extends almost to the ventral sucker where it branches into two short crura. A conspicuous eyespot consisting of a mass of coarse, dark brown granules is located on each side of the pharynx.

The ganglionic mass, observable in specimens stained with neutral red or sev-

eral other *intra vitam* stains, proceeds laterally from the region of the pharynx to the eye spots where it branches into anterior and posterior rami. The genital primordium, which stains light pink with neutral red, is represented by a large variously-shaped mass of cells lying dorsal to the ventral sucker. It may extend from the anterior or central regions of the sucker to well beyond its posterior edge. The thick-walled excretory bladder extends forward almost to the ventral sucker. The cells constituting the bladder are large and are filled with globules that stain deeply with neutral red. The flame cells number 24, the formula being 2 [(2+2+2) + (2+2+2)]. Some 20 spherical, deeply staining cystogenous glands are located on each side of the cercaria, from just in front of the oral sucker to the posterior end of the body.

Ten cercariae killed in hot 10 per cent formalin measure: body length, 0.200–0.230 mm. (av., 0.211 mm.); body width, about 0.091 mm.; tail length, 0.330–0.350 mm. (av., 0.342 mm.); oral sucker 0.042 by 0.032 mm. to 0.046 by 0.040 mm. (av., 0.044 by 0.037 mm.); ventral sucker, 0.032 by 0.032 mm. to 0.039 by 0.039 mm. (av., 0.036 by 0.036 mm.); pharynx, about 0.018 by 0.016 mm.; eyespots, about 0.014 by 0.014 mm.; stylet, about 0.023 by 0.008 mm.

A comparison of cercaria *C. cornutum* and cercaria *C. cooperi* indicates that they are much alike. The chief difference lies in the nature and arrangement of the cells of the excretory bladder. There is no orderly arrangement of large-nucleate cells in the bladder wall of cercaria *C. cornutum* or a muscular-walled outlet duct as depicted in cercaria *C. cooperi.* Hopkins also described two masses of cells lying in a tandem position ventral to the excretory bladder as representing the testes. No such structures were observed in cercaria *C. cornutum.*

The cercariae readily penetrate the crayfish host and encyst only in the cardiac region. Encystment occurs within a few hours. Examination of metacercariae ten days after infection showed that they still possessed stylets and eyespots, that the excretory bladders were large and conspicuous because of the accumulation of many refractile granules, that the oral sucker was moderately lobed and the prepharynx much shortened. The intestinal ceca had grown to the posterior region of the body. Metacercariae attain full size within 6 to 8 weeks.—Donald J. Ameel, *Department of Zoology, University of Michigan.*

A REDESCRIPTION OF *DRACUNCULUS MEDINENSIS**

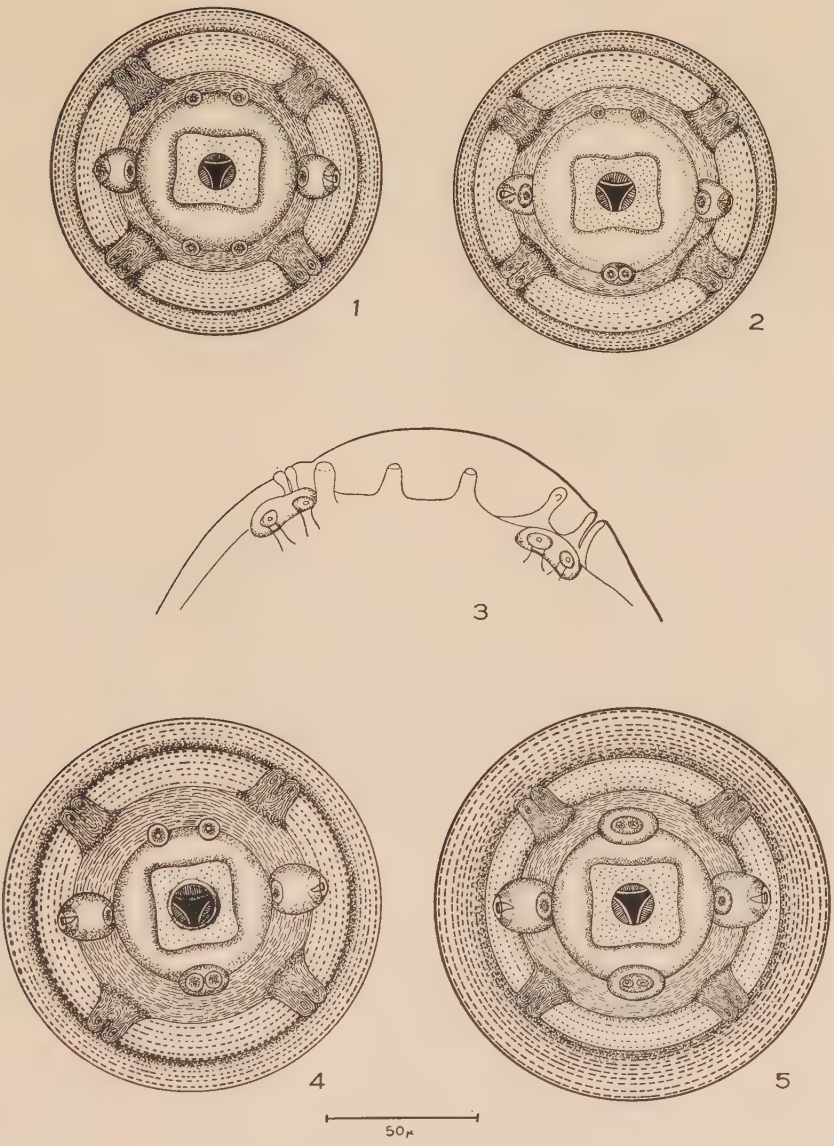
Moorthy and Sweet (1936, Ind. Med. Gaz., 71: 437–442) reported on the experimental infection of dogs with *D. medinensis* and gave a preliminary description of stages (including males) encountered. To date a total of 6 pups have become successfully infected by feeding them with *Cyclops* containing larval *D. medinensis* and 45 males and 178 females have been recovered after periods of 67 to 457 days. The following table summarizes the results obtained:

* From the Department of Helminthology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

Acknowledgment is made to the International Health Division of the Rockefeller Foundation for having granted the fellowship which made this work possible.

The writer is greatly indebted to Drs. P. A. Maplestone, P. Parthasarathy, and W. C. Sweet for the helpful criticism and advice given during the preliminary stages of this work in India. To Dr. B. Schwartz the writer's appreciation is due for the privileges of his laboratory and to Dr. B. G. Chitwood and Mrs. M. B. Chitwood, the writer wishes to express his gratitude for the very valuable help and guidance given in the course of this work.

PLATE I



Dracunculus medinensis

Fig. 1. Male head (en face view)—interno-dorsal and interno-ventral pairs of papillae separate.
Fig. 2. Male head (en face view)—interno-dorsal papillae—separate; interno-ventral papillae—partially fused.
Fig. 3. Male head—median view.
Fig. 4. Immature female head—length 24 mm. (en face view).
Fig. 5. Mature female (en face view).

PLATE II

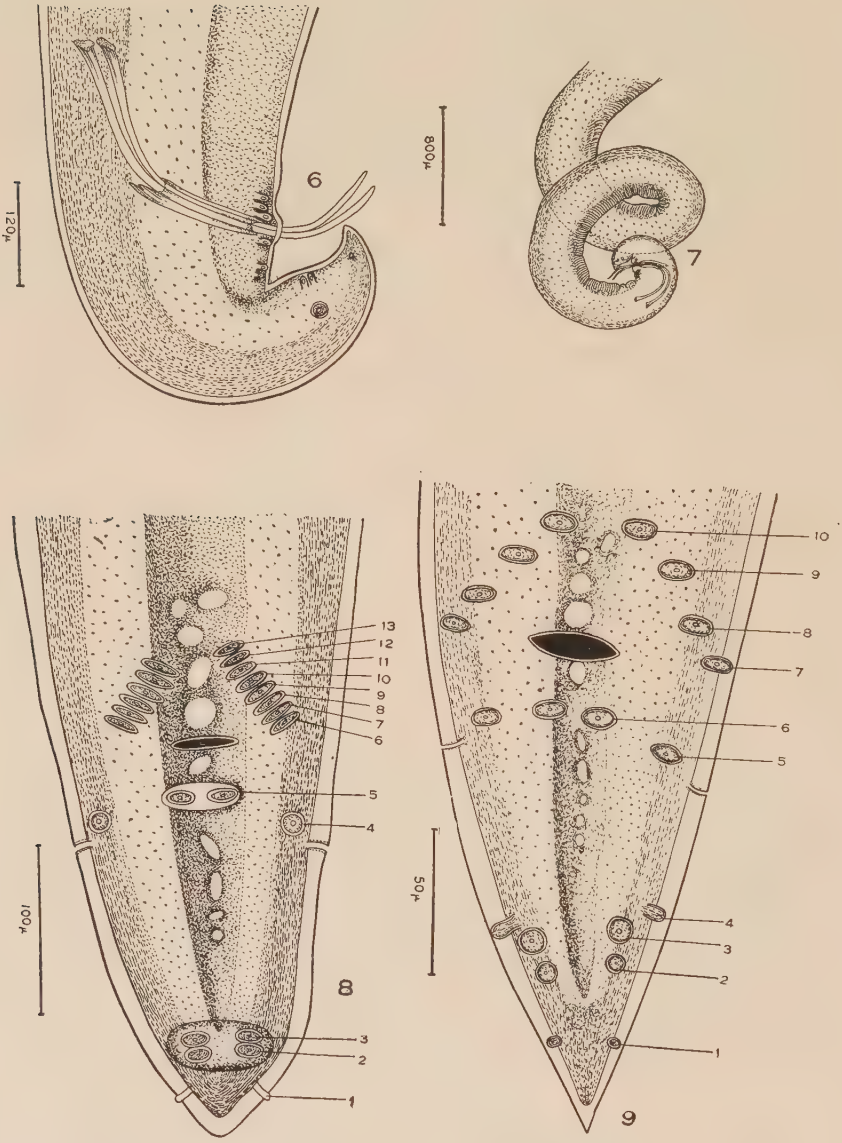


Fig. 6. Male tail (*Dracunculus medinensis*)—lateral view.
Fig. 7. Male tail (*Dracunculus medinensis*)—general view.
Fig. 8. Male tail (*Dracunculus dahomensis*)—ventral view.
Fig. 9. Male tail (*Dracunculus medinensis*)—ventral view.

PLATE III

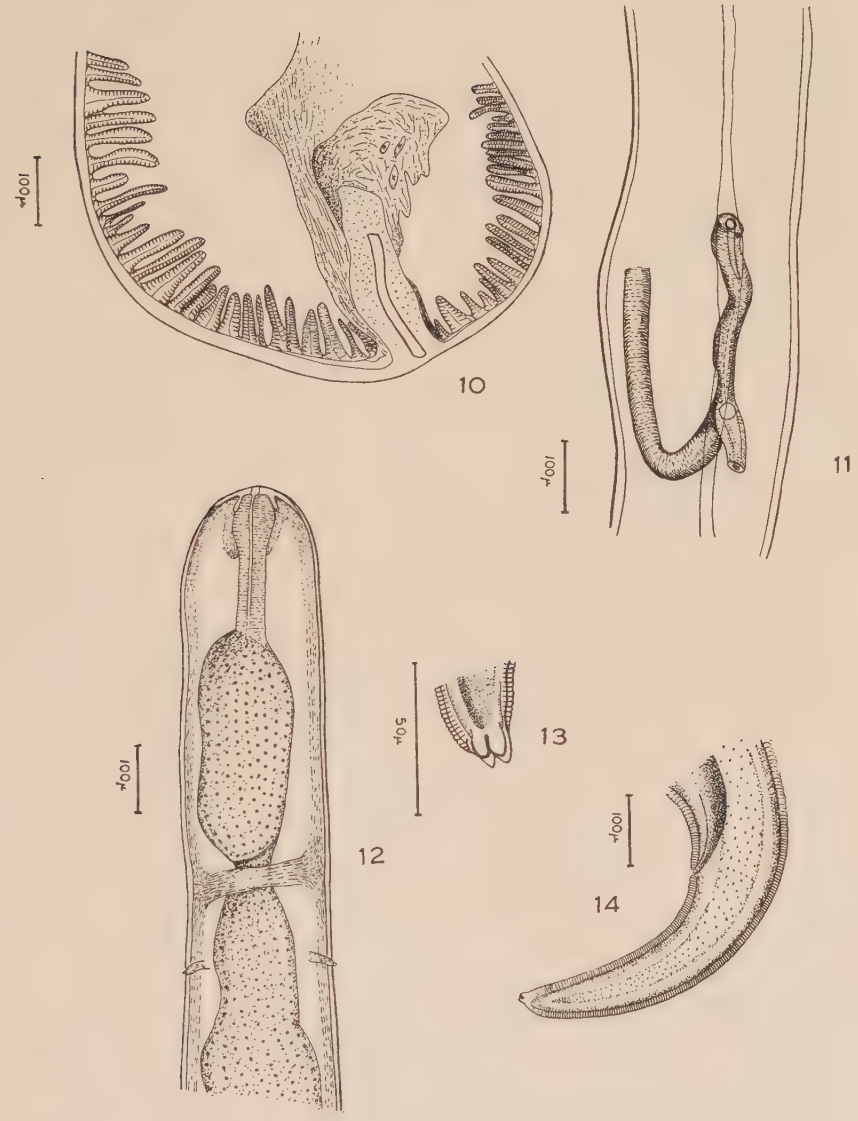


Fig. 10. Cross section of mature female (length 31.5 cms.) in the region of the vulva showing the presence of mucoid plug.
Fig. 11. Female (length 24 mm.)—shows the vulva, vagina containing mucoid plug, and origin of uteri.
Fig. 12. Male—oesophageal region.
Fig. 13. Immature female tail (length 24 mm.) showing four caudal mucrones.
Fig. 14. Immature female (length 24 m.m.) showing the position of anus.

	Dog No. 28	Dog No. 19	Dog No. 20	Dog No. 23	Dog No. 21	Dog No. 14
No. of days after administration of infected *Cyclops*...	67	105	115	133	144	457
Number of males	7	5	17	9	7	0
Number of females	44	1	74	23	18	21
Length of males (in cms.) ..	1.2–1.8	1.8–2.4	2.0–2.2	2.1–2.9	2.0–2.9	.....
Length of females (in cms.).	1.5–2.4	18	17–33	2.5–50	9–45	28–53
Larvae present	No	No	No	No	No	Yes

The following description of *Dracunculus medinensis* has been made from the study of this material.

Male.—12 to 29 mm. long by 0.4 mm. wide; oral opening minute, surrounded by an internal circle of 4–6 well-developed papillae (interno-laterals always separate, interno-dorsals and/or interno-ventrals sometimes partially fused) and an external circle of 4 double papillae (no variations); amphids posterior to interno-laterals (Figs. 1, 2, and 3); deirids (cervical papillae) slightly posterior to nerve ring (Fig. 12); oesophagus 9.6 mm. long in specimen 26 mm. long, consisting of a short anterior muscular part and a wide posterior glandular part constricted at isthmus where nerve ring is situated (Fig. 12); anus 250 μ from posterior extremity; genital papillae 10 pairs; 6 postanal and 4 preanal; numbering from the posterior extremity, pair 1 ventro-lateral; pairs 2–3 subventral, not circumscribed; pair 4 ventro-lateral near 3 and separated some distance from pairs 5–6 which are postanal, 5 being ventro-lateral and 6 subventral; 7–10 in a series from ventro-lateral to subventral just anterior to anus; phasmids lateral, posterior to pair 5. Spicules subequal, 490–730 μ long; gubernaculum 200 μ long (Figs. 6, 7 and 9).

Female.—1.5 to 53 cms. long by up to 1 mm. wide; quadrangular circumoral elevation present; oral opening surrounded by an internal circle of 4 papillae; interno-laterals separate, interno-dorsals and interno-ventrals partially fused; one large female had interno-ventrals separate; small females often similar to males (Fig. 4); external circle of 4 double papillae; amphids posterior to interno-laterals (Figs. 4 and 5); oesophagus 10 mm. long in specimen 24 mm. long. Dr. B. G. Chitwood found oesophagus 40 to 60 mm. long in specimens 75 cms. long obtained from North American mammals. Intestine pigmented in young specimens, gradually losing pigmentation with maturity. Anus 250 μ from posterior extremity in specimens 24 mm. long (Fig. 14) 900 μ in specimens 50 cm. long; tail with four caudal mucrones in young specimens (Fig. 13), without such structures in fully grown specimens. Vulva 10.3 mm. from anterior end in specimens 24 mm. long (Fig. 11). Dr. B. G. Chitwood found the vulva to be situated 14.5 cm. from the anterior end (Fig. 10) in specimens 31.5 cm. long. Vagina containing mucoid plug in specimen 24 mm. long illustrated (Fig. 11).

Apparently copulation takes place in the earlier stages of development since males were not observed after six months and the vagina contains a mucoid plug in young specimens (24 mm. long).

Dracunculus dahomensis (Neuman, 1895) is very difficult to distinguish from *D. medinensis* on the basis of the females. However, the arrangement of the genital papillae and the shape of the tail of the male (Fig. 8) provide quite satisfactory characters for the separation of these species. In this species papillae 2–3 are circumscribed; papillae 4 corresponds to 5 of *D. medinensis;* and 5 (6 of *medinensis*) is also circumscribed. The preanal papillae are apparently variable with 6–8 pairs, sometimes not all paired. *D. globocephalus* (Mackin, 1927), differs in that the spicules are markedly unequal. *D. houdemeri* Hsu, 1933, was based on a single female, the distinguishing characteristic being the cephalic pattern, the lateral cuticular elevations being trident-like. Since variations of the cephalic pattern are known in this genus, the validity of the species appears dubious.—V. N. Moorthy, *Fellow of the Rockefeller Foundation from Mysore State Department of Health, Bangalore, India.*

HAEMOGLOBIN IN A LARVAL NEMATODE

Mueller (1934, Bull. New York State Coll. Forestry, 7: 356–373), described immature worms belonging to the genus *Eustrongylides* from *Fundulus diaphanus menona,* which were found in fibrous capsules attached to the mesenteries and which were blood-red. Apparently the same parasite was found by Mr. F. F. Bond in *Fundulus heteroclitus* and this material was used for the present investigation.

The red color of the worms is due to the coelomic fluid, in which the pigment is dissolved. This pigment is readily water soluble, but insoluble in ether and turns brown when heated in a boiling water bath. After drying and heating with sodium chloride and acetic acid dark brown crystals were observed, which were somewhat greater than those resulting from similarly treated fish blood. The crystals were readily soluble in 5 per cent KOH solution. Their characteristics are therefore those of hemin crystals. The body fluid of some worms was collected and diluted with about one cc. of distilled water. A dilution of fish blood was prepared of about the same color intensity. Both solutions were compared spectroscopically and showed the same typical bands of oxyhaemoglobin. After addition of Stokes's reagents, an identical broad band characteristic for reduced haemoglobin was visible in both tubes. After shaking the solution of reduced worm pigment with air, the bands of oxyhaemoglobin reappeared. The red pigment present in these worms is, therefore, doubtless haemoglobin. Small worms seem to contain much less haemoglobin than older ones, but a worm never was found totally without it. There is no indication as to whether this haemoglobin is derived from the blood of the host, or whether it originates in the worms themselves. Owing to the fact that the haemoglobin is apparently present in relatively high concentration, it is assumed that it is not without biological significance. Many larval helminths have an oxidative metabolism, and it might be that the haemoglobin of this worm is present in order to secure oxygen under the difficult respiratory conditions under which it lives. The above findings are reported here briefly, since this seems to be the first case of haemoglobin in a larval parasitic worm.

In adult helminths haemoglobin has, so far the author could ascertain, only been reported from *Ascaris lumbricoides* and *Parascaris equorum* by Flury (1912, Arch. exp. Path., 67: 273–290), Fauré-Fremiet (1913, C. R. Soc. Biol., 74: 567-569) and Krüger (1936, Zool. Jahrb., Abt. Allg. Zool. u. Physiol., 57: 1–56). It seems to be present here in small amounts only. Magath (1919, Trans. Amer. Micro. Soc, 38: 43–170), stated that the coelomic fluid of *Camallanus americanus* is reddish and he assumed that it contains some part of the pigment group of the blood of the host. He was, however, unable to get hemin crystals. His worms lost their reddish color after remaining some time in water, whereas the color of the present form was unchanged after living for 48 hours in saline at 37° C.

The writer is indebted to Mr. F. F. Bond for help in securing the material and to Dr. A. M. Baetjer for help during the spectroscopical work.—Th. v. Brand, *Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.*

EFFECT OF STARVATION ON TRICHOMONADS IN RATS

Conditions in the lumen of the intestines can be modified easily by starvation and in certain instances the effects of such changes on intestinal protozoa have been observed. For example, Cleveland found that if the large Pacific termite, *Termopsis angusticollis,* was starved for 6 days it lost its infection with the intestinal flagellate *Trichonympha* and if it was starved for 8 days it lost a second type of intestinal flagellate, *Leidyopsis.* It is also well known that when frogs are brought into the laboratory and kept without food they tend to lose many of their intestinal protozoa, probably because of starvation.

Laboratory rats are ordinarily heavily infected with trichomonad flagellates

which live principally in the cecum. Six rats with good infections were selected from our colony and kept in a cage with a screen bottom through which their fecal pellets dropped immediately on passage. They were given as much water as they wished to drink, but no food, for 8 days. At the end of this time they were killed and examined. The cecum of every rat was only about one-fifth the size of that of a well-fed rat. The cecal contents were soft and evidently consisted principally of digestive secretions. All the rats were infected with trichomonads; one infection was considered very heavy, three heavy, one fair and one light. Evidently trichomonads are not easily eliminated from rats by depriving the host of food. Whether they would disappear before the host died of starvation is still to be determined.—Robert Hegner and Lydia Eskridge, *School of Hygiene and Public Health, Johns Hopkins University.*

THE HATCHING OF SPORES OF *NOSEMA BOMBYCIS* NÄGELI AND THE PARTIAL DEVELOPMENT OF THE ORGANISM IN TISSUE CULTURES

In an attempt to obtain the development of the microsporidian parasite, *Nosema bombycis,* in silkworm tissue cultures of the type successfully used in the cultivation of the virus of grasserie (Trager 1935, J. Exp. Med, 61: 501–513), the following observations were made.

1. Emergence from the spore. As is well known, spores may be readily induced to hatch by feeding them to silkworms of any age. If a drop of digestive fluid is removed from the gut of a caterpillar about half an hour after the insect has ingested spores, the hatching of the spores can be observed in a hanging drop. Filament extrusion takes place with a violent corkscrew motion. At the end of the filament there usually appears a spherical mass of clear protoplasm, as observed by Ohshima (1927, Annot. Zool. Japon., 11: 235–243). This slowly becomes smaller and finally is lost to sight. Kudo (1916, Bull. Imp. Seric. Exp. Sta., 1: 31–51) was unable to obtain hatching of the spores *in vitro* (under physiological conditions, filament extrusion may of course be induced by acid, strong salt, H_2O_2, etc.) even if they were placed in digestive fluid removed from normal silkworms. Ohshima, on the other hand, obtained hatching under these conditions. I have found that the spores in a suspension in Ringer's solution kindly given me by Dr. Ohsima regularly hatched when placed in hanging drops of silkworm digestive fluid and behaved like spores fed to a silkworm and subsequently removed and observed. But spores obtained from insects infected with Ohshima's spore suspension completely failed to hatch under the same conditions, and the same was true of all my other spore suspensions obtained from insects infected with a strain sent me by Prof. A. Paillot. All these spore suspensions infected silkworms just as readily as Ohshima's suspension, but only the latter hatched *in vitro.* Perhaps the fact that Ohshima's suspension was considerably older than any of the others accounts for this discrepancy. The spores of Ohshima's suspension did not hatch in silkworm blood, yeast-extract, trypsin, various salt solutions, etc., but they did hatch almost as well as in silkworm digestive fluid when placed in 5% Eli Lilly Co. liver extract no. 343 at a pH of 8 or higher. It is significant that at a pH below 7 very few of the spores hatched. The alkaline liver extract was an excellent medium for the observation of hatching. The extruded filaments and the protoplasmic spheres at their distal end remained visible a long time. Giemsa staining of such preparations showed that the protoplasmic spheres were minute binucleate organisms exactly corresponding to the "amebulae" of Stempell (1909, Arch. f. Protist., 16: 281–358).

2. Development of the parasite in tissue culture. Amebulae hatched out *in vitro* from spores of Ohshima's suspension could not be inoculated into tissue cultures because this spore suspension contained bacteria. The spores in numerous suspensions prepared aseptically from infected pupal or larval tissues could not be induced to hatch *in vitro.* Accordingly, the following procedure was employed. About 0.02 cc. of a very heavy spore suspension from infected pupae was fed to

each of 3 nearly full-grown silkworms. The following day one of the larvae was bled aseptically from the proleg and 0.005 cc. of blood was added to each of 2 cultures of silkworm ovarian tube lining cells. On the next day, the other two larvae were bled aseptically, their blood pooled, and 0.005 cc. added to a tissue culture. No *Nosema* ever appeared in either of the first two cultures. In the culture inoculated with blood from larvae 2 days after their infective feeding, on the 10th day after inoculation, several of the wandering cells and an outgrowing strand of tissue were observed to contain numerous spores and sporonts of *N. bombycis*. Wandering cells full of parasites were still motile and were as active as uninfected cells. Although many similar experiments have been performed, no other case of development of the parasite in tissue culture has been obtained, probably because too few infective forms were present in the blood used. The one successful case proves unequivocally the presence, in the blood of silkworms which have recently ingested spores, of minute extracellular infective forms, the "planonts" of Stempell, the proof for the existence of which has been doubted (Kudo) and has heretofore rested solely on morphological evidence.—WILLIAM TRAGER, *Department of Animal and Plant Pathology, Rockefeller Institute, Princeton, New Jersey.*

SOME NOTES ON THE EFFECT OF ATEBRINE ON THE GAMETOCYTES OF THE GENUS *LEUCOCYTOZOON*

O'Roke (1934, Univ. Mich. Sch. Forestry and Conservation, Bull. No. 4), working with *L. anatis* in ducks, seems to be the only worker to report experiments to test the effect of drugs on a *Leucocytozoon*. This author used quinine sulphate, plasmochin and quinine dihydrochloride, and concludes that these drugs are of no avail against the adult gametocytes.

It was decided to test the effect of Atebrine on this genus of blood parasites when two birds, a great horned owl (*Bubo virginianus*) and a juvenile red-tailed hawk (*Buteo borealis*) came under observation during the summer of 1935. Both birds were carrying rather heavy infections with a spindle-shaped *Leucocytozoon*, and in addition, the owl had a heavy infection with an halteridium of the *Haemoproteus columbae* type.

In carrying out the treatment the drug was dissolved in distilled water and given *per os*. The owl received 140 mg. doses each day for 7 days; then the treatment was discontinued for 2 days and repeated on the 10th day. Although the bird did not show any evidences of drug intoxication, it died on the 11th day. The hawk was treated with 100 mg. doses each day for 7 days; then a 2-day rest period was allowed, followed by 5 days of treatment with 140 mg. doses.

Smears were made daily and stained with Wright's. The effects of the drug are largely reported in terms of morphological changes produced in the parasite or host cell. These changes largely involved the appearance of clear vacuoles in the parasite and host cell, those in the latter containing purple staining bodies of various sizes. We have been unable to agree as to the nature of these bodies and have, therefore, decided to designate them as "granules" in this note.

The owl.—The first change in the female gametocytes was noted on the 3rd day of treatment when small vacuoles, containing small irregular-shaped granules, appeared in the host cell near the parasite. On the 4th day numerous vacuoles appeared in the parasite. Large vacuoles with granules were present near the pointed ends of the host cell. The 5th day the irregular-shaped vacuoles in the host cell contained 5–6 granules, sometimes clustered together. On the 8th day the parasites were definitely reduced in number and those remaining had vacuoles measuring up to $3\,\mu \times 3.6\,\mu$. The following day as many as 12 vacuoles, the largest measuring $2.9\,\mu \times 4\,\mu$, with rod-shaped granules were present in the host cell. Smears made on this day showed some parasites that had separated from their host cells. No particular change was noted on the following day, except that the number of parasites was greatly reduced. The male gametocytes gave much the same reaction to the drug with the exception that a few showed small vacuoles with their contained granules on the 2nd day of treatment.

The *Haemoproteus* parasites, mentioned above, showed morphological changes which very closely paralleled the results previously reported by the senior author for *H. columbae* of the pigeon's blood (1935, Am. Jour. Hyg., 21: 249–259).

The hawk.—The female gametocytes showed numerous small vacuoles on the 3rd day and generally one vacuole, containing a small irregular granule, at the ends of the host cell. The next day the vacuoles in the parasite were more numerous. Those in the host cell were larger and each contained an ellipsoidal granule. By the 5th day the large vacuoles in the host cell contained up to three granules. On the 6th day larger vacuoles measuring up to 1.7 μ were present in the parasite and little change was noted in the host cell. The 7th day, vacuoles in the parasite measured up to 2.9 μ. Larger granules appeared in the vacuoles of the host cell on the following day. The 8th day found little change in the parasites, except that in a few cases up to two vacuoles were present in the nucleus. The host cell vacuoles measured up to 2.2 μ. The following day the vacuoles in the parasite and host cell measured up to 3.8 μ. During the next two days the parasites began to elongate and a decided drop in the total parasite count was noted. On the 16th day no female gametocytes could be found in the blood.

In the main, the male gametocytes reacted in much the same way as did the females, except that some of them had vacuoles with their contained granules by the 2nd day of treatment. These vacuoles increased in size more rapidly than those in the female parasites. On the 9th day the granules, some not surrounded by vacuoles, measured 1 μ × 1.4 μ. By the 12th day these granules measured 1.4 μ × 2.6 μ. Although the total parasite count fell rather rapidly after the 13th day, there were a few male gametocytes still present in the blood on the 16th day when the bird was dropped from further observation.

The data presented here would indicate that Atebrine does have some parasiticidal effect on the *Leucocytozoon* parasite, but whether it could be used effectively for the control of *Leucocytozoon* disease can only be determined by further experimentation.

We wish to thank the Nebraska Academy of Sciences for financial aid and the Winthrop Chemical Company, Inc., of New York for supplying the Atebrine used in these experiments.—G. Robert Coatney and Evaline West, *State Teachers' College, Peru, Nebraska.*

VITAMIN A–DEFICIENT DIET IN RATS IN RELATION TO INFECTION WITH *E. MURIS*

In attempting to infect Vitamin A-deficient rats with *E. histolytica* it was noted that the A-deficient animals were free from *E. muris* infection, whereas the control rats on the normal diet were usually infected. The Vitamin A-deficient diet, which was furnished to us by Doctor K. E. Mason, of the Department of Anatomy, was made up of the following constituents: casein, 18 per cent; corn starch, 50 per cent; lard, 20 per cent; Wesson's salt mixture, 4 per cent, and brewer's yeast, 8 per cent. The normal diet for control rats consisted of Purina dog biscuits and city tap water.

Further studies were devised in order to determine the relation of vitamin A-deficiency to infection with *E. muris*. It was found that rats fed the above A-deficient diet could not be infected by feeding *E. muris* cysts. The same group of rats after two weeks on the normal diet were again fed *E. muris* cysts and almost all became infected. Normal rats infected with *E. muris* lost their infection when placed on the A-deficient diet. Supplementing the A-deficient diet with cod-liver oil, substituting dextrin for corn starch, the addition of powdered agar to increase the bulk, and the addition of granulated sugar (sucrose) to encourage the passage of softer fecal pellets, failed to furnish an environment necessary for the maintenance of *E. muris* infection. These experiments demonstrated that the lack of vitamin A was not the cause of the inability to establish or maintain infection with *E. muris*.

Doctor Mason then prepared an A-deficient diet by extracting powdered dog

biscuit for seven days with 95 per cent alcohol. When rats which had been A-deficient on the casein diet were placed on the extracted dog biscuit diet they continued to show physical signs of A-deficiency. These rats were fed *E. muris* cysts and infection was established in all the rats in the group. Cysts appeared in the stools in from 3 to 9 days after the last feeding. Autopsy examination one month after the cysts were fed showed motile forms of *E. muris* in the cecum of all the rats, although they all still showed marked evidence of A-deficiency.

These experiments demonstrated that neither lack of vitamin A in the diet nor the condition of A-deficiency in the rats rendered the lumen of the cecum unsuitable for the development of *E. muris*.—William W. Frye and Henry E. Meleney, *Department of Preventive Medicine and Public Health, Vanderbilt University School of Medicine, Nashville, Tennessee.*

THE PRODUCTION OF AMOEBIC LIVER ABSCESSES IN CATS THROUGH THE PORTAL CIRCULATION

In attempting to produce sterile amoebic liver abscesses in cats, cultures of *Endamoeba histolytica,* from which about 99 per cent of the bacteria had been removed by washing with sterile Ringer's solution, were injected directly into the portal vein following laparotomy. Forty-six cats, half-grown or larger, were employed and the livers examined in from 3 to 10 days after injection of the amoebae. Two cats, one killed six days and one seven days after inoculation, each had a single abscess located just beneath the superior surface of the caudal division of the right lateral lobe. *E. histolytica* and bacteria were found in the scrapings from both abscess cavities. Microscopic sections in each case showed amoebic invasion of the liver tissue.

Cleveland and Sanders (Amer. Jour. Hyg., 12: 569–605) were able to produce amoebic abscesses in the liver of cats by injecting *E. histolytica* directly into the liver tissue, but they were unable to produce abscesses in 8 cats by injection into the mesenteric vein.

Most authors are agreed that in man liver abscesses are produced by the transportation of the amoebae through the portal circulation from the primary focus in the intestine. As far as we know, this report is the first experimental demonstration of the production of amoebic liver abscesses by way of the portal circulation.—William W. Frye, Arnoldo Gabaldon, and Henry E. Meleney, *Vanderbilt University School of Medicine, Nashville, Tennessee.*

A MICROSPORIDIAN INFECTION OF *FUNDULUS HETEROCLITUS* (LINN.)

A collection of *Fundulus heteroclitus* (Linn.) was taken October 3, 1935, from Chesapeake Bay at Baltimore, Md., and placed in an aquarium. About a week later one fish (7.5 cm. long) exhibited a slight swelling about 1 mm. above the surface on the left side of the body anterior to the dorsal fin above the lateral line, and posterior to the pectoral fin. This was only visible from above and no difference in superficial appearance from the surrounding surface was noted. Throughout this period of two weeks this fish was fed daily with liver and evidenced no inconvenience or lack of appetite due to the steadily increasing size of the swelling which remained localized although raised .3–4 mm. above the surface of the body. The enlargement was set off by raised scales and a whitening of the edges of the scales over and about the edges of the spot. The portion of the fish infected was cut away and the whole piece fixed in Bouin's.

Sections stained with Harris's hematoxylin and eosin showed general oedema and muscle degeneration with much infiltration near the center of the infected tissue. This gradually decreased in intensity toward the edge of the infected tissue until cellular infiltration and distorted muscle layers were the only evidences of the disturbance. Throughout most of the sections the oedema remained general. The muscle degeneration did not include every muscle fiber but most of the fibers

near the center showed some destruction, while a few were completely destroyed and the remaining sarcolemma was filled with phagocytic cells and granular debris. Within the remaining sarcolemma of the most degenerate fibers or in the nearby interfibrillar spaces were found small masses (Fig. 1, A) containing typical micro-

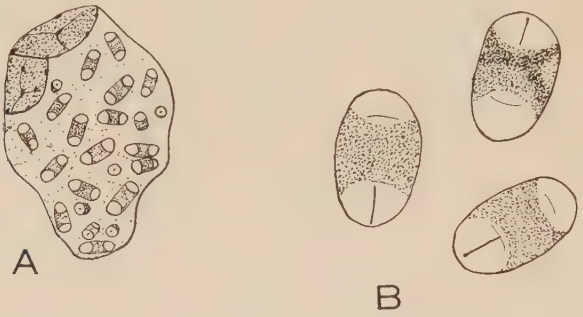


FIG. 1.—*Plistophora* sp. from *Fundulus heteroclitus* (Linn.). A. Sporont with large nucleus. ×3000. B. Spores from sporont. Free-hand drawing. Iron hematoxylin.

sporidian spores (Fig. 1, B). Further study was carried out with material stained by Giemsa's technique, iron hematoxylin, or Wright's triple connective-tissue stain.

The parasite masses were found to contain an irregular number of spores, 10 to 18 having been counted in various masses. These spores were oval and measured 2.5–3 by 1.5–2 microns in length. The sporoplasm was situated as a band about the middle of the spore while at each end of the spore was a vacuole. The sporont or pansporoblast varied in size from 18–30 microns in diameter. These consisted of a fine envelope and often could be related to a large nucleus, although just as often this could not be located. The "pansporoblastic nuclei" in a number of cases were similar to the structure of the surrounding tissue nuclear forms. There was no cyst formation in any case and the parasites were more or less diffusely scattered throughout the interfibrillar spaces and the degenerate muscle tissues. This lack of cyst formation together with the production of a large and irregular number of spores places this form in the genus *Plistophora* Gurley of the family *Nosematidae* Labbe.

A survey of the literature revealed 27 species of Microsporidia infecting fish. Of these, 9 species are reported to infect muscle tissue. In the genus *Plistophora* two species, *P. destruens* Delphy (1916, C. R. acad. sci., 163: 71–73) and *Plistophora* sp. Drew (1909, Parasitol., 2: 193–194), are of special interest. The pathology of these two species and that described above are of nearly the same form. Drew's form gives an almost identical picture although there is no mention of a tumerous swelling. Both Drew and Delphy fail to give figures of the spore form so that comparison except in general form and measurements is impossible. The present form agrees with the measurements of the form described by Delphy, but the spore and pathological picture correspond to that of Drew. The size of the spore as given by Drew, 2.5 to 3 microns in length (it has been noted in Kudo, 1924, Ill. Bio. Mono. IX (2 & 3): 179, that these dimensions were stated as "not given"), might have been more nearly like that of the present parasite since Drew's material was not well kept and the dimnsions of the present form were based on sections.—FRANKLYN F. BOND, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

MYXOSOMA GRANDIS KUDO IN FISH FROM THE HUDSON RIVER DRAINAGE SYSTEM

The material upon which this note is based was collected during the summer of 1936 while with the Stream Unit of the New York State Conservation Survey. In four lots of fish preserved in 4 per cent formalin were found specimens with the abdominal distension characteristic of infection with *Myxosoma grandis* Kudo, originally described from the silver-mouthed minnow, *Ericymba buccata* (Cope), from the Salt Fork of Vermilion River, Illinois (Kudo, R. 1934, Ill. Biol. Monog. XIII (1) : 15). The description of parasites and pathology accompanying the infection as given by Kudo are very similar to those recorded here. Three species of minnows, *Notropis hudsonius amarus* (Girard), *Notropis hudsonius hudsonius* (Clinton), and *Rhinichthys atronasus atronasus* (Mitchill) were found to be infected by this parasite. I am indebted to Dr. John Greeley and other members of the New York State Conservation Survey of 1936 for identifications and collection of part of the above material.

The specimens of *Notropis h. amarus* were from two general locations, the Rifton Reservoir situated on the lower Wallkill River and in the Wallkill River itself at a point about 5 miles down stream from Walden, New York. The presence of infections at two such points would probably indicate that the greater portion of the stream harbors infected fish. The incidences in two collections taken in Rifton Reservoir August 11, 1936, were 24 of 43 positive and 12 of 24 positive as determined by abdominal distension. The incidence was probably much higher since infections not causing distension were missed. The parasites were normal in form and size; all mature spores of normal form were well within the limits of the measurements given by Kudo (length 15–16 μ, breadth 9–11 μ, thickness 6–8 μ, polar capsules 6–7 μ by 2–3 μ). Liver pathology in section presented a picture of many vegetative forms, 50–150 μ in diameter, and in varying stages of development, with only a small portion of the liver remaining on one edge. The whole liver was sectioned in four cases and the scarcity of liver tissue remaining in these suggested that the host could not have lived much longer. In three cases the infection was not confined to the liver alone, but parasites were located in the muscle tissue beneath the peritoneum, and could easily be seen from the external surface as white masses since only a thin layer of epidermis and muscle covered the surface. These masses were 5–8 mm. in diameter and 1–2.5 mm. thick and generally caused no external swelling. A few infected fish were received from the second location in August and when the same location was visited in early September more infected fish were found. These infected fish moved much more slowly than others of the same group, and once seen could be readily caught.

Three infected specimens of *Notropis h. hudsonius* were received from a collection made by the River Unit of the New York State Conservation Survey. These were taken August 25, 1936, at a point in the Hudson River about 4½ miles north of Haverstraw, New York. Two fish, 4.6 and 4.8 cm. in length, showed definite abdominal enlargement while the third (10.3 cm. long) had only a light infection. One cyst about 3 mm. in diameter was found in the anterior lobe of the liver of the latter. Infection was similar to that of *Notropis h. amarus,* the spores being normal and of the same general dimensions.

The specimens of *Rhinichthys a. atronasus* came from a small stream, the Jan de Bakker's Kill (which is a tributary of the Hudson River by way of Catskill Creek). The infected fish were found at a point about 3¾ miles from Cairo, New York. On the first collection, late in June, it was reported that five such enlarged fish were seen although only two were preserved. In September two more infected specimns were taken. At this time a large number of fish of all species present in the stream were collected but no added infections were found. The infected fish (4.0, 4.2, 4.3, and 2.5 cm. long) were suffering from extreme distension of the abdomen. The liver tissue was almost totally destroyed and the fish that were seen alive swam only with the greatest of difficulty. The parasites presented a much different picture from those noted above. Many irregu-

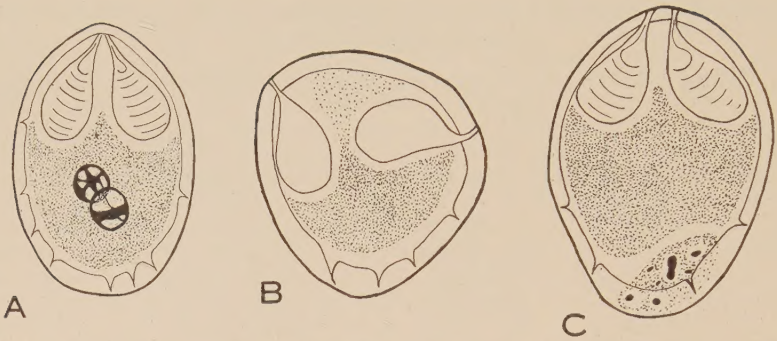


FIG. 1.—A. *Myxosoma grandis* Kudo, normal spore from *Notropis h. amarus*. Harris's hematoxylin, formalin material. B and C. abnormal spores from *Rhinichthys a. atronasus*. Fresh preparation. ×2100.

larities in spore shape were seen and only a small percentage of the mature spores corresponded to those originally described and to those previously noted. Elongation, rounding or actual distortion occurred in most of the spores in which polar filaments had developed. A large percentage of these had misplaced polar capsules. The greater severity of infection and the numerous abnormalities in spore shape suggest that this host is not as favorable for the development of the parasite as the other three species of fish mentioned above. The figure included here shows a normal spore from *Notropis h. amarus* (Fig. 1, A), and two abnormal spores (B and C) from the liver of *Rhinichthys a. atronasus*.—FRANKLYN F. BOND, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

REVIEWS

AN INTRODUCTION TO NEMATOLOGY. Section I; part I. By B. G. Chitwood and M. B. Chitwood. Monumental Printing Co., Baltimore, Md., 53 pp., 53 figs., 1937. Price $2.00.

When the reviewer began graduate work in parasitology in 1905, there were parasitologists who knew something of nematodes parasitic in animals, and nematologists, as Cobb called them, who knew something of free-living nematodes and nematodes parasitic in plants, but there was no one with sound and high standing in either group of workers who had any comparable standing in the other group. The classification of animal-parasitic nematodes took cognizance of such parasites only, aside from the unavoidable cases of *Rhabditis* and its relatives, and the classification of free-living and plant-parasitic nematodes showed a similar exclusiveness aside from the same unavoidable exceptions. For many years, the late Dr. N. A. Cobb protested, in print and at many meetings of the Helminthological Society of Washington, that such a situation was unsound, and that a sound classification would provide major groups which individually would contain animal-parasitic, free-living and plant-parasitic nematodes.

In time the reviewer became convinced that Dr. Cobb was right, but it was evident that such a classification could not be made in any adequate fashion until it was made by someone with sufficiently extensive first-hand knowledge of the major groups of nematodes in general to speak with some authority on the subject. Some years ago, the reviewer was in a position, administratively, to assign someone to such a project in the Federal Bureau of Animal Industry, and at that time Dr. B. G. Chitwood was available. He had had several years' experience with free-living and plant-parasitic nematodes in the Federal Bureau of Plant Industry in association with such able workers as Cobb, Steiner and Christie, and had the background of sound training in zoology in Asa Chandler's laboratory. With such preparation and what amounted to a fixation on the subject of nematodes, Dr. Chitwood was assigned the task of familiarizing himself with animal-parasitic nematodes, and preparing, on that basis and his existing background, a classification of nematodes in general.

Dr. Chitwood has developed such a classification and, over and above this, has become one of the best authorities on nematodes in the world. He and his wife, M. B. Chitwood, have now begun the publication of this monographic series entitled "An Introduction to Nematology." Part I of Section I states, in the preface, that the work is written from a strictly zoological standpoint as opposed to an economic, medical, veterinary or purely taxonomic standpoint. This first publication of the series opens with a historical review of nematology. This is followed by a chapter of general discussion of the structure of nematodes, including an outline classification of the major groups of nematodes with the more significant and characteristic structures and a general discussion of the groups. The two succeeding chapters deal, respectively, with the external cuticle and hypodermis, and with the somatic musculature, connective tissue, body cavity, and organs of body cavity. The publication closes with an outline classification given in some detail and without discussion. The authors place the class Nematoda in the phylum Aschelminthes of the subkingdom Vermes. Readers who are familiar with the older classification will find many new groups and new groupings in this publication. Undoubtedly, exception will be taken to some of the proposals, and this will stimulate more critical consideration of all classifications of nematodes, which is one of the first steps to better taxonomy.

The chapters on morphology are particularly good, and are well illustrated. Although the numbering system indicates that there are only 53 figures, there are actually about 380 figures. The bibliographies on each topic are adequate for the purposes of the book. The cover design is very attractive and striking. Altogether it is an excellent piece of work.

So far as can be ascertained from a reviewer's reading (the usual somewhat hasty reading of the whole, with more careful reading of "samples"), the publication is relatively free from misprints, and the creation of only one synonym by misspelling was noted. However, the reviewer would urge a more careful editing of future sections of this work to make sure in all cases that the ideas in the heads of the authors were actually put into words that would convey the same ideas to the reader. The authors succeed very well in this in the field of morphology, but in their general discussion we note the following: "Nematodes have habitats more varied than have any other group of animals save the arthropods, and even this group is foreshadowed by the immense numbers of individual nematodes. Yet of this massive assembly that live and thrive over the whole earth, few are ever heard of by others than nematologists and even these are rarely seen." Just what is meant by these baffling sequences of ideas and this selection of words is not at all clear to the reviewer. In a general way we get the idea that there are a lot of nematodes, but there is a flavor of *non sequitur* and general improbability about the wording. These sentences constitute an isolated case, not at all representative of the publication in general, but the reviewer would like to see such vagaries eliminated from subsequent sections for the benefit of readers and the protection of the authors from reviewers. M. C. HALL.